THE EFFECT OF HYDROXYLAMINE ON THE GROWTH OF WHEAT

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In the standard scheme of nitrate reduction in plants, hydroxylamine certainly acts as an intermediate product which, according to this diagram is further reduced to ammonia:

$$HNO_3 \rightarrow HNO_2 \rightarrow (NOH)_2 \rightarrow NH_2OH \rightarrow NH_3.$$

It is generally accepted that ammonia is precisely the compound which is involved in amino acid synthesis in plants. However, even as far back as the end of the last century, Bakh [1], and also Meyer and Shulze [2], expressed the hypothesis that hydroxylamine may participate directly in the synthesis of amino acids. In that case the diagram presented above takes on the following form:

$$HNO_3 \rightarrow HNO_2 \rightarrow (NOH)_2 \rightarrow NH_2OH \rightarrow NH_3.$$
$$\searrow \quad \swarrow$$
amino acids

According to this hypothesis the hydroxylamine formed in plants reacts with carbonyl compounds and forms oximes, which when reduced are transformed into the corresponding amino acids.

However, up to the present time, there have been no clear indications received that hydroxylamine may be assimilated and participate in amino acids synthesis in plants. Furthermore, it has been established that hydroxylamine formed by the cell nucleus inhibits a whole series of processes, especially the reaction of fermentive transamination [3].

Wood and Hone [4] studied the possibility of the assimilation of hydroxylamine and various oximes by oat plants in unsterile water cultures. The results which the authors obtained permitted them to maintain that hydroxylamine and especially oximes are involved in the synthesis of organic nitrogenous substances in plants.

It is quite clear that the question concerning the possibility of hydroxylamine participation in the nitrogen metabolism of plants should above all be studied using appropriate concentrations; at low concentrations plants are able to assimilate hydroxylamine, at high concentrations it acts as a cellular poison. Actually, we showed [5] that at certain concentrations of hydroxylamine an intensive accumulation of glutamic acid and serine occurs in homogenized as well as living plants. In addition, in experiments with a simultaneous addition of hydroxylamine and α-ketoglutaric acid to homogenized and living wheat and squash seedlings as a substrate for transamination, we discovered that at certain corresponding concentrations of ketonic acid and hydroxylamine the latter is the source of the amino group in the synthesis of glutamic acid and serine. At high concentrations, however, hydroxylamine induces a concurrent retardation of the transamination reaction of amino acids with α-ketoglutaric acid [6].

At the same time we investigated the biochemical processes during the assimilation of hydroxylamine by plants; we also studied the effect of hydroxylamine on the growth and development of young wheat plants.

METHODS

Seeds of summer wheat, variety Moskovka, were used in the investigation; these were soaked in water for seven hours and then distributed on moist paper. After roots and shoots appeared the seedlings were placed on grids which had been treated with paraffin and in which an opening was left for the roots; the grids were then placed over grooves filled with water so that the roots were submerged in water and then left under natural light conditions. After seven days (in most of the experiments) the green seedlings were carefully removed so as not to injure the roots, and used in the experiments. The hydroxylamine ($NH_2OH \cdot HCl$) solution, brought to a pH = 6 by the addition of $NaHCO_3$, was introduced into the living seedlings by means of its absorption through the roots with the transpiration current in one series of experiments [7], and by means of vacuum infiltration in another [8].

1. Introduction of Hydroxylamine into the Seedlings with the Transpiration Current

In one series of experiments the roots of 25-28 seedlings were placed in vials with solutions (pH = 6) of various concentrations of hydroxylamine (0.0001; 0.001; 0.006; 0.025; 0.1; and 0.2 M) for six hours, or in another series of experiments into hydroxylamine in an equimolar mixture with α-ketoglutaric acid (0.0001; 0.001; 0.006; 0.0125; 0.025; 0.05M). Plants with their roots immersed in water served as controls. After this the plants were removed from the vials, dried with filter paper, and quickly planted into wooden boxes containing garden soil. After 24 hours, when the plants had recovered

TABLE 1. The Effect of Hydroxylamine, Introduced by Absorption Through the Roots, on Wheat Seedlings in Soil*

Concentration of NH_2OH (M)	On the fourth day of growth in the soil			On the seventh day of growth in the soil		
	average length of plants ± probable error, cm (M ± m)	standard deviation (σ)	coefficient of variation (cv)	average length of plants ± probable error, cm (M ± m)	standard deviation (σ)	coefficient of variability (cv)
Water	17.9 ± 0.94	1.37	7.7	22.1 ± 0.51	2.1	9.8
0.0001	16.5 ± 0.37	1.55	9.4	21.0 ± 0.54	2.2	10.8
0.0010	14.6 ± 0.27	1.30	8.9	20.1 ± 0.55	2.7	13.2
0.0050	13.2 ± 0.24	1.18	9.0	18.3 ± 0.59	2.5	14.0
0.0250	6.0 ± 0.40	1.70	28.0	12.9 ± 0.92	3.8	29.0
0.0500	3.8 ± 0.14	0.59	15.5	5.3 ± 0.32	1.3	24.0
0.1000	4.5 ± 0.47	2.0	4.4	Died		
0.2000	Died			Died		

*The five days of the seedlings growth were counted from the day the seeds were soaked; the length of the seedlings at the time of planting into the soil was 3-4 cm.

TABLE 2. The Effect of a Mixture of Equimolar Amounts of Hydroxylamine and α-Ketoglutaric Acid Introduced Through the Root System on the Growth of Wheat in Soil*

Conc. NH_2OH + α ketoglutaric acid (M)	On the third day of growth in the soil			On the sixth day of growth in the soil		
	average length of plants ± probable error, cm (M ± m)	standard deviation (σ)	coefficient of variability (cv)	average length of plants ± probable error, cm (M ± m)	standard deviation (σ)	coefficient of variability (cv)
Water	11.3 ± 0.36	1.21	10.7	22.0 ± 0.31	1.3	5.9
0.0001	13.9 ± 0.02	1.00	7.2	22.8 ± 0.47	2.0	8.7
0.0010	11.8 ± 0.16	0.65	5.5	22.3 ± 0.27	1.3	5.8
0.0060	11.8 ± 0.02	1.00	8.4	21.7 ± 0.28	1.2	5.8
0.0125	11.0 ± 0.24	1.00	9.1	21.9 ± 0.50	2.1	9.9
0.0250	8.0 ± 0.21	0.80	10.3	21.5 ± 0.37	1.5	7.3
0.0500	10.7 ± 0.19	0.80	7.5	20.2 ± 0.36	1.7	8.3

*The five days of the seedlings growth were counted from the day of soaking; the length of the seedlings at the time of planting into the soil was 3-3.5 cm.

from the transplanting, we began to measure the length of the shoots and observe the general condition of the plants. The experiments were terminated 15-20 days after the plants had been transplanted into the boxes. The measurements obtained were analyzed statistically.

Table 1 contains the results of length measurements on the fourth and seventh days of growth in the soil for one typical experiment in which hydroxylamine was introduced.

The data in Table 1 show that when hydroxylamine at a concentration of 0.0001 was drawn up into the root system for six hours, there was a slight inhibitory effect upon further growth of the plants; as the concentration was increased this effect became more pronounced, however, the external appearance of the plants was no different from that of the controls. Observations showed that on the 12th day the experimental plants became equalized, their growth approached that of the controls, and at hydroxylamine concentrations of 0.0001 and 0.001 M they even exceeded the size of the controls.

The results of the experiment in which a mixture of hydroxylamine and α-ketoglutaric acid in equimolar concentrations was introduced into the root system are given in Table 2.

From Table 2 it is evident that when equimolar amounts of hydroxylamine and α-ketoglutaric acid were introduced into the plants by means of absorption by the root system, at concentrations of 0.0001 and 0.001 M, a noticeable stimulation of growth was observed, but at concentrations of 0.006 and 0.0125 M the experimental plants were practically the same as the controls. Even at concentrations of 0.025 and 0.050 M, which re-

TABLE 3. The Effect of Vacuum Infiltration of Hydroxylamine on the Growth of Wheat Plants in the Soil *

Concentration of NH_2OH (M)	On the third day of growth in the soil			On the fourth day of growth in the soil		
	average length of plants ± probable error, cm (M±m)	standard deviation (σ)	coefficient of variability (cv)	average length of plants ± probable error, cm (M±m)	standard deviation (σ)	coefficient of variability (cv)
Water	12.6±0.64	2.88	22.8	14.1±0.76	3.26	23.1
0.0001	14.3±0.53	2.56	17.9	16.2±0.75	3.60	22.2
0.0010	13.3±0.42	2.08	15.6	16.2±0.50	2.10	12.9
0.0100	12.0±0.43	2.20	18.3	15.6±0.48	2.08	13.3
0.1000	Died	–	–	–	–	–

* The age of the seedlings was six days, counting from the day the seeds were soaked; length of the seedlings at the time of planting into the soil 6-7 cm.

tarded plant growth when hydroxylamine alone was used, satisfactory growth of the plants was observed when hydroxylamine and α-ketoglutaric acid were introduced simultaneously.

2. Introduction of Hydroxylamine into the Seedlings by Vacuum Infiltration

In order to check the possibility of using vacuum infiltration for solving physiological problems in living plants in our first experiment (vacuum infiltration of water) we used small seedlings on their fourth day of growth (length of the seedlings – 4 cm). However, the experiment showed that after the seedlings had been planted into the boxes with soil, the plants died very quickly. Therefore the following experiments were done with six-day-old plants (length of seedlings – 6 cm). These experiments were successful. We found the boat-shaped glasses (Fig. 1) 13-14 cm long, 2.5 cm wide, and 2.5 cm deep, which we used, to be especially suitable in which to arrange the plants for vacuum infiltration; they made it possible to carry on the vacuum-infiltration without injuring the plants, and with a minimum amount of infiltrating solution. Plants of this size withstood careful vacuum infiltration of water very well, developed roots quickly when they were transplanted into the soil, and started to grow vigorously.

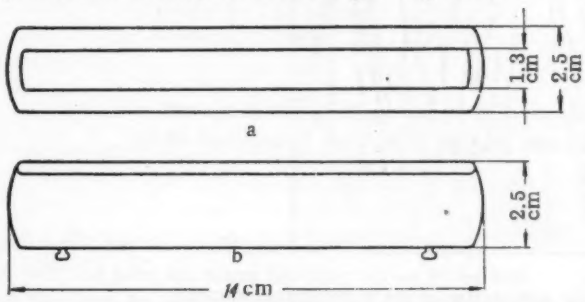

Fig. 1. Boats for vacuum infiltration of seedlings. a) Top view, b) side view.

This showed that in those instances when it is necessary to use the living seedlings further in physiological experiments, vacuum infiltration should be performed with more mature seedlings (no less than 5-6 days after soaking the seeds). In some of our further experiments, the growth of the plants which had been subjected to vacuum infiltration with pure water even exceeded that of plants not subjected to vacuum infiltration.

Our experiments showed quite clearly that vacuum infiltration of young plants and their subsequent cultivation in the soil or in nutrient solutions can be used as a method for "forced" introduction of various metabolites and physiologically active substances into the plant with the object of studying various physiological and biochemical problems in the plants.

After our preliminary experiments we went over to vacuum infiltration experiments with seedlings using hydroxylamine solutions of various concentrations. In all we performed eight such experiments; they all gave practically the same results. Therefore, we are presenting the results of only two of these.

Vacuum infiltration was performed as we described above with maximum care to guard against any injury to the plants during all the manipulations with them and especially when air was admitted into the vacuum-exsiccator. After infiltration of the plants was completed they were still left in the vacuum exsiccator for 20 min and were then removed from the solutions, dried slightly with light applications of filter paper, and then planted immediately in boxes with soil which were kept in the greenhouse; measurements of growth and observations on the condition of the plant were made daily from the time the plants were planted in the soil, over a period of two weeks and longer.

The results of one of these experiments are given in Table 3.

The results given in Table 3 show a definite stimulation of growth of wheat at low concentration of hydroxylamine (0.0001 and 0.001 M).

The results of another similar experiment are shown in Table 4.

Table 4 shows that in this experiment low concentrations (0.0001 M) slightly retarded the growth of plants. In spite of this, the plants did not differ in their external appearance from that of the controls either in color or width of the leaves. It is interesting to note that upon further growth, the growth of these plants exceeded that of the controls (Fig. 2).

When equimolar amounts of hydroxylamine and α-ketoglutaric acid were introduced into the plants by vacuum infiltration a noticeable weakening of hydroxylamine toxicity was observed, just as it was in the experiments where such a mixture was absorbed through the roots. Even at concentrations of 0.0125 and 0.025 M, no toxic effect was observed, and furthermore, there was some stimulation of plant growth. This is apparent from the data given in Table 5.

We also performed four experiments in which the endosperm was removed from the plants following vacuum infiltration with hydroxylamine before they were planted into the soil. All these experiments yielded corresponding results. The results of one of these experiments is given in Table 6.

These data show that wheat seedlings rooted very well and developed further, even when the endosperm was removed following vacuum infiltration with hydroxylamine.

In such instances on the fourth day of growth in the soil the seedlings which had been infiltrated with solutions of hydroxylamine in concentrations of 0.0001, 0.001 and even 0.01 M developed better than the con-

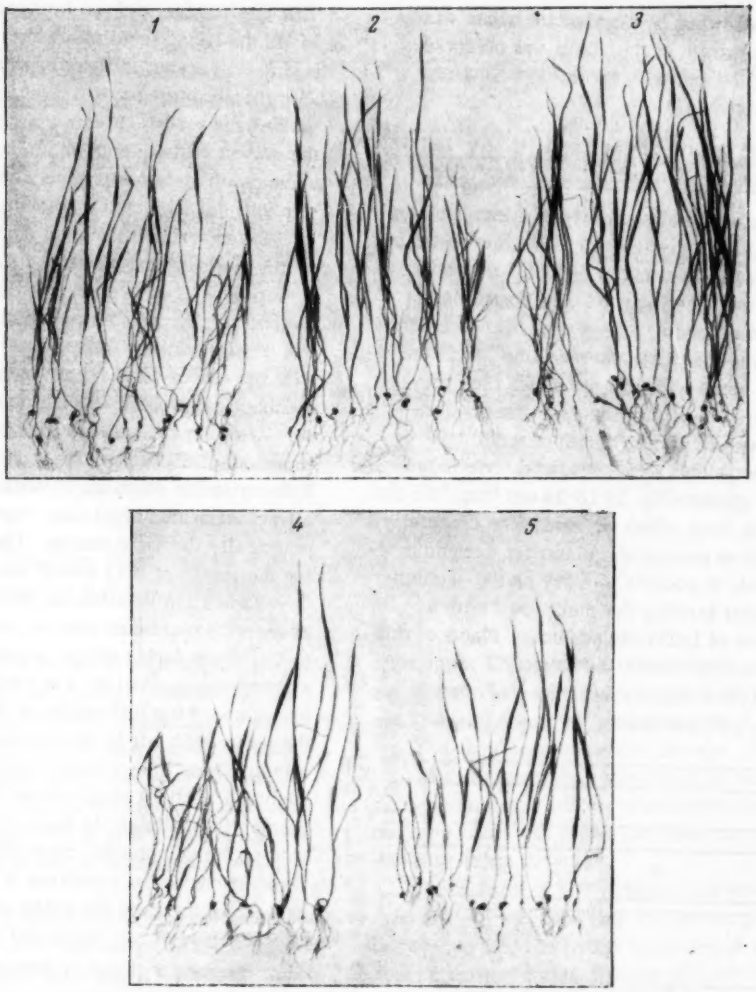

Fig. 2. View of dried plants following completion of the experiment. Vacuum infiltration of the seedlings with hydroxylamine in the following concentrations (M): 1) H_2O; 2) 0.0001; 3) 0.001; 4) 0.01; 5) 0.0025.

TABLE 4. The Effect of Hydroxylamine Vacuum Infiltration on the Growth of Wheat Plants in the Soil •

Conc. of NH_2OH (M)	On the third day of growth in the soil			On the seventh day of growth in the soil		
	average length of plants±probable error, cm (M±m)	standard deviation (σ)	coefficient of variability (cv)	average length of plants±probable error, cm (M±m)	standard deviation (σ)	coefficient of variability (cv)
Water	18.0±0.38	1.97	10.9	18.9±0.63	3.30	17.4
0.0001	16.2±0.36	1.90	11.8	18.3±0.50	2.50	13.9
0.0010	13.9±0.32	1.68	12.1	17.8±0.44	2.30	12.9
0.0100	14.5±0.31	1.64	11.2	17.0±0.35	1.87	11.1
0.0250	11.9±0.39	1.52	12.7	15.2±0.38	1.45	9.5

• The age of the seedlings was seven days, counting from the day the seeds were soaked; length of the seedlings at the time of planting into the soil [no value given in original].

TABLE 5. The Effect of Vacuum Infiltration of Equimolar Amounts of Hydroxylamine and Ketoglutaric Acid on the Growth of Wheat•

Conc. of NH_2OH and α-ketoglutaric acid (M)	On the third day of growth in the soil			On the seventh day of growth in the soil		
	average length of plants±probable error, cm (M±m)	standard deviation (σ)	coefficient of variability (cv)	average length of plants±probable error, cm (M±m)	standard deviation (σ)	coefficient of variability (cv)
Water	13.5±0.26	1.40	8.5	20.2±0.46	2.5	12.3
0.0125	13.5±0.20	1.10	10.0	22.3±0.33	1.7	7.9
0.0250	13.0±0.30	1.70	8.0	20.6±0.48	2.6	12.5
0.0500	11.9±0.42	1.80	13.6	19.7±0.47	2.5	12.7
0.1000	Didn't grow	—	—	11.0±1 12	3.6	32.3

• The age of the seedlings was six days, counting from the day the seeds were soaked; length of the seedlings at the time of planting into the soil 8-9 cm.

TABLE 6. The Effect of Hydroxylamine Vacuum Infiltration on the Growth of Wheat Plants in the Soil Following the Removal of Their Endosperm after Vacuum Infiltration•

Conc. of NH_2OH (M)	4th day of growth in soil (1st leaf)			7th day of growth in soil (2nd leaf)		
	average length of plants±probable error, cm (M±m)	standard deviation (σ)	coefficient of variability (cv)	average length of plants±probable error, cm (M±m)	standard deviation (σ)	coefficient of variability (cv)
Water	13.5±0.20	0.97	7.2	14.0±0.36	1.7	12.6
0.0001	15.4±0.24	1.20	7.9	17.0±0.45	2.2	13.1
0.0010	15.4±0.28	1.30	8.9	17.0±0.51	2.5	14.7
0.0100	14.9±0.33	1.60	11.0	13.3±0.66	3.2	24.1
0.0250	13.0±0.23	1.10	8.4	12.7±0.45	2.1	16.7

• The age of the seedlings was 10 days, counting from the day the seeds were soaked; length of the seedlings at the time of planting into the soil 10-11 cm.

trols which had been infiltrated with water. On the seventh day after the plants had been set out in the soil, we measured the second developing leaves and found that their length in these variants also surpassed that of the controls (Table 6).

Therefore, it is apparent from all the given data that low concentrations of hydroxylamine had a stimulating effect on the plants, or else only a slight inhibitory effect on the growth of the plants without affecting their external appearance. However, at higher concen-

trations of hydroxylamine a progressive toxic effect was observed. When hydroxylamine was introduced into the plants simultaneously with α-ketoglutaric acid, there was a marked decrease of its toxicity and a definite stimulation of plant growth. Hence, the data which we obtained indicate that hydroxylamine, especially in oxime form can be readily assimilated by the growing organism and is actually an intermediate product in the reduction of nitrates.

The data which we obtained during a study concerning the transformation of hydroxylamine in living tissues and homogenized plants also indicate this [9]. These results show that in the plant, hydroxylamine undergoes enzymatic transformation, accompanied by the formation of ammonia, amino acids, and small amounts of hydroxamic acid.

In addition, these data verify Pryanishnikov's statement [10] that "the problem of toxicity is conditional, – it all depends on the concentrations in question".

SUMMARY

The effect of hydroxylamine on the growth and development of wheat shoots was investigated. Hydroxylamine in concentrations of 0.0001, 0.001, 0.006, 0.0125, 0.025, and 0.05 M was introduced into 6-10-day-old seedlings by two methods: 1) by suction through the root system together with the transpiration current and 2) by vacuum infiltration. In a number of experiments hydroxylamine was introduced in an equimolar mixture together with α-ketoglutaric acid. The seedlings were then immediately planted in a box with soil. After 3 and also 6-7 days after planting, growth of the seedlings was measured. The data obtained were treated statistically.

When the vacuum infiltration of the seedlings was carried out in special boat-shaped glasses the plants satisfactorily endured introduction of the solution and after the treatment excellently took root and developed further. This method may thus serve as a convenient method of "forced" introduction of various metabolites and physiologically active substances in plants for the purpose of studying various problems of plant physiology and biochemistry.

It was shown that small concentrations of hydroxylamine stimulated growth of the seedlings, especially during later stages of development. High concentrations of hydroxylamine exerted a progressively increasing toxic effect. When hydroxylamine was introduced together with α-ketoglutaric acid, stimulation of growth appeared at a much higher concentration than if only hydroxylamine was introduced. Even at such high concentrations, which led to a more or less pronounced inhibition of growth when only hydroxylamine alone was introduced, the seedlings were found to develop satisfactorily. It was thus shown that hydroxylamine can be fixed by plants, and thus is an intermediate product in the reduction of nitrates in plants.

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*See English translation.

THE EFFECT OF SURPLUS MOISTURE ON PLANTS AT DIFFERENT DEVELOPMENTAL PERIODS

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Until recently the problem of plant reaction to differences in soil moisture concerned principally the study of effects on agricultural products of moisture shortage in their surrounding environments, i.e., drought.

The problem of the effect of excessive soil moisture on plants, especially on annual plants, has been little investigated [1-8].

However, such an investigation is of great theoretical and practical importance to regions with excessive soil moisture or to regions with periodically occurring excessive moisture phases (for example, to podzol, peaty soils of our northern, northwestern regions and Far-Eastern regions), as well as to irrigated regions.

Investigation of the effect of excessive soil moisture on plants in relation to their development, i.e., at different periods of their ontogenesis, was complete neglected. This would also be of great importance to irrigated agriculture. For instance, in irrigation by inundation, the lowland parcels are inevitably excessively moist, deteriorating the soil structure and diminishing the plant yield. Also a difference in plant relationship to autumn and spring inundation of winter cultivations (soaking) has been established [9], which can be explained variously, particularly by their differing phase and age stages.

In connection with the above, for a number of years (1954-1959), we have investigated the effect of excessive soil moisture on grains (barley, wheat) and recently also on potatoes at different periods of their development. The investigation of this problem will assist in establishing the upper, yet optimal, limits of soil moisture for plants, as well as the degree of moisture resistance of plants in general and during different periods of their development in particular.

This article is a brief report of our experiments on grain cultures (two barley varieties).

The experiments were conducted in vegetational pots. The plants were cultivated at soil moistures of 60-70, 100, and 120% of total moisture capacity during the entire growth period. Soil moistures of 100 and 120% were applied also by periods of development, the stage of which we determined by morphological changes at growth points of the main stem.

The effects of different moisture levels on crop structure, selected physiological processes, metabolism, and pollen vitality were investigated.

Participating in this study were our co-workers and fellow-candidates (P. I. Lerman, N. N. Verzilin, N. N. Savitskaya, A. Z. Aleksandrova, and Yu. N. Fedorova). Only a portion of the data obtained is published in this article.

The studies showed that different barley varieties are quite resistant to high soil moisture (100% of total moisture capacity), irrespective of their preference for dry or moist soils. A sharp decline in yield occurs only with excess moisture during the entire growth period. Excess moisture in periods from sprouting to formation of the first ears in the cone of growth of the main stem (vernalization stage), and from the formation of incipient ears to formation of the pollen in maternal cells and tetrads from them (light stage), causes a considerable decline of yield, differing in different varieties, depending on their nature. In a number of cases greater resistance is shown to excess moisture in periods from sprouting to formation of incipient earing on the growth cone of the main stem (Tables 1, 3).

In comparing results by varieties, a similar effect of abundant soil moisture on plants is noted in the period from end of the light stage to earing (positive action) as well as in the period of light stage (negative action). The differences in action of abundant soil moisture on the yield of barley plants (Viner and Pallidum varieties) in the vernalization stage and light stage can be explained by the fact that the Viner variety is more adapted to moist soils than the Pallidum variety. The latter is more affected by an abundance of moisture. Abundant soil moisture for plants from the end of the light stage to earing, i.e., during the critical period, increases the general grain yield.

Weather conditions of 1955 and 1957 were good. Results of the effects of 100% soil humidity on plants, given in Tables 1 and 3, were obtained in growth periods of moderate temperature environments and fairly high relative humidity. The experiments at the above soil moistures were repeated by us several times over a number of years. The results were in good agreement, indicating that 100% soil moisture capacity, with rare exceptions, does not greatly diminish the yields of barley grains.

The plants behaved somewhat differently at this soil moisture in the environment of the exceptionally dry and hot summer of 1959. Weakened by high soil mois-

TABLE 1. Effect of Abundant Soil Moisture on Barley Varieties Pallidum and Viner (1955 experiment)

Weight of grains	Pallidum				Viner			
	100% humidity			60% humidity	100% humidity			60% humidity
	vernalization stage	light stage	critical period		vernalization stage	light stage	critical period	
From the entire plant (g)	23.4	1.8	33.1	23.8	32.2	22.9	41.1	27.5
Same, as % of control	98.0	76.0	139.0	100.0	117.0	83.0	149.0	100.0
From the main stem (g)	16.2	14.1	17.5	16.7	13.0	11.2	13.1	12.1
Same, as % of control	87.0	84.0	105.0	100.0	107.0	92.0	108.0	100.0

TABLE 2. Effect of Abundant Soil Moisture on Viner Barley Variety (1959 experiment)

Weight of grains	100% humidity			70% humidity
	light stage	third stage	critical period	
From the entire plant (g)	11.1	9.8	12.6	22.1
Same, as % of control	50.3	44.5	57.0	100.0
From the main stem (g)	5.9	6.1	7.1	7.6
Same, as % of control	77.6	80.3	93.4	100.0

ture, they were, of course, affected to a greater degree in a dry year than in moist years. However, the general mechanisms for this soil humidity were preserved. The least decrease in yield was observed during the critical period, and along the main stem in the third* stage of development (Table 2).

The results of all our experiments in studying the effects of abundant moisture on plants confirm the hypothesis we expressed at one time on the great need of water by plants during the period of laying foundations for maternal cells of pollen and tetrads and also during fertilization. During this period, when plants enter the gametophyte phase, there is a need for high soil moisture and high hydration of plant tissues [11].

Flooding (120% of total moisture capacity, see Table 3) has a different effect. Flooding after seeding produces sparse, uneven sprouts, indicating a negative effect of excessive soil moisture during grain germination; in some pots the sprouts were either inhibited or totally absent.

Table 3 (for comparison, data on the effect of 100% soil moisture are shown) indicates that the greatest damage occured in plants flooded during the entire growth period. They were in a depressed condition and at the end of growth produced a very low yield. All the physiological processes in these plants are in a lowered state, while their anatomic structure displays a clearly xeromorphic character.

Flooding at the vernalization stage decreases the final yield to a lesser degree than at other stages of development, since the plants manage to recuperate by the end of growth. Flooding at the light stage and the third stage (as per V. A. Novikov) markedly decrease growth processes, shown by lessening the growth in height and a decreased area of assimilating surface to $^1/_3$ to $^1/_5$. The root system's growth is also decreased. Thus, for instance, determinations of the root system's volume conducted after recuperation produced the following results: control – 78 cm^3; flooding at light stage – 18 cm^3; flooding at the third stage – 25 cm^3. Flooding also acts negatively on root absorption of the basic elements of root nutrition (N, P, K). Flooding at these stages increases the amounts of bound water and decreases the amounts of free water in leaf tissues, and also decreases

*The third stage of development was evolved by V. A. Novikov [10], who conceives its beginning as the moment of staminal tubercle appearance and its end the appearance of tetrads in anthers.

TABLE 3. Effect of Abundant Moisture and Flooding on Barley Yield of Viner Variety (g per 10 plants)

Experimental variants	1957		1957		1958	
	g	%	g	%	g	%
Control (moisture 70%)	23.6	100.0	23.6	100.0	29.5	100.0
	Moisture	100%	Flooding		120 %	
Total period of growth	15.9	67.3	3.9	16.7	2.0	6.8
Vernalization stage	19.8	83.8	18.6	78.7	—	—
Light stage	21.5	91.1	7.9	33.6	9.9	33.4
Third stage (per V. A. Novikov)	—	—	13.1	55.3	14.7	49.6
Critical period	22.8	96.5	23.9	101.0	23.4	79.2

TABLE 4. Effect of Flooding on Water Content (%) and Osmotic Pressure (atm) of Cell Juice in Viner Barley Leaves

Experimental variants	Total Hydration	Bound water	Free water	Osmotic pressure
Control (70% moisture)	84.2	14.1	70.1	7.16
Flooding in light stage	80.1	37.9	42.1	10.30

total hydration (Table 4). At the same time there is an increase in osmotic pressure of cell juices in leaf tissues and a reduction in transpiration intensity to $^1/_2$-$^1/_3$.

On flooding, the activity of some oxidative enzymes, particularly peroxidase, are increased, indicating increased respirational activity, probably a result of aeration deficiency in the root zone in these conditions (Karishnev [1]).

Flooding at the critical period still does not exert such a deeply destructive effect on plants compared to other periods of development. The content of reduced ascorbic acid, approaching the control, and a relatively large percentage of viable pollen (see Table 6) indicate that the viability level of plants flooded at the critical period is quite high. The preservation of the aqueous regime and growth processes in experimental plants on a level close to the control secures a normal development in experimental plants, and after renewal of normal conditions in the soil's water supply, will yield a sufficiently high crop. However, on careful analysis of obtained data, some increase in the respiratory enzymes' activity and a decrease in absorption of the basic elements of mineral nutrition from the soil is found. This latter circumstance indicates that even in critical periods, flooding leaves it traces on plants.

In the hot and dry summer of 1959 the pattern of plant behavior at different periods of their ontogenesis remained approximately the same; however, the unfavorable influence of excessive moisture was more marked than in the moist years. This evidently is due to the weakened condition of the experimental plants by comparison with the control. Also, as in the moist years, the greatest effect is evident in periods of generative organ formation (Table 5).

Investigating the effects of flooding on pollen viability (determinations were conducted by the Shardakov method and fluorescent microscopy) we found the same conformity with the rule – the percentage of fertile pollen decreases on flooding in the same sequence as plant yield (Table 6).

In the dry summer (1959) in connection with the greater damage to plants, we also discovered a certain parallelism between plant yield and pollen viability. When the greatest decrease in yield was noted (the third stage), the greatest damage to pollen occured.

It is also important to note that the decrease in pollen viability, which correlates with the decrease in yield, is related to disruption of the whole course of microsporogenesis. Flooding caused disruption in processes of reduction-division. In the anaphase the division of chromosomes was delayed; frequently they improperly moved to wrong poles. Anomalies were also observed in the telophase of the first reduction division as well as in the second division. Frequently dyads, and even binuclear cells were formed at such times instead of tetrads. The appearance of these anomalous phenomena, the same as in drought action [12, 13, 14] was related to an increase in protoplasm viscosity. The nonviable pollen formed on flooding was frequently found to be greatly deformed.

Disruption of microsporogenesis and formation of sterile pollen, as well as the decrease in plant yield on flooding, are related to disruption of metabolism (carbohydrate and protein). These investigations con-

TABLE 5. Effect of Soil Flooding on Viner Barley (1959 experiment)

Weight of grains	Vernalization stage	Light stage	Third stage	Critical period	Soil moisture 70 %
From the entire plant (g)	10.98	6.1	0.4	3.9	22.1
Same, as % of control	49.7	27.5	1.7	17.4	100.0
From the main stem (g)	6.8	3.4	0.4	3.1	7.6
Same, as % of control	89.5	44.7	5.3	40.8	100.0

TABLE 6. Effect of Flooding on Pollen Viability (%)

Experimental data	Control moisture 70%	Flooding			
		entire period	light stage	third stage	critical period
Annual mean	95.4	61.1	83.0	76.1	86.3
In 1959 (dry and hot year)	94.0	—	94.9	0.9	69.0

tinue in the realm of metabolic studies. It is possible that the formation of sterile pollen on flooding is not the only cause of the decrease of the final yield.

Excessive moisture and flooding cause numerous manifestations in the soil which sharply change the conditions of plant growth and their development, which in periods of high soil moisture is much prolonged.

There is a basis for thinking that the cause for negative activity of excessive moisture and soil flooding are: disruption of soil structure, its aeration; the disruption of the normal proportions between soil oxygen, and carbon dioxide leaning toward an increase of carbon dioxide. This inhibits not only the activity of the root system, but also the normal progress of such biological processes which provide soil fertility [15].

There is an undoubted similarity between plant behavior at excessive moisture (especially when flooded) and water lack in the soil. It is manifested both in the over-all plant reaction to these conditions and in the changes of the aqueous regime in the course of one or another period of its development, and also in metabolic changes, growth processes, etc.

The study of excess moisture or soil flooding effects on plants is undoubtedly very important. Therefore, further investigations in this area with different agricultural products is desirable.

SUMMARY

1. Barley is relatively resistant to soil moisture at 100% total moisture capacity. The tested varieties in temperate weather environments usually reacted positively to the above moisture during growth periods at vernalization stage and in the critical period, but negatively in the light stage. The yield decreased sharply only when this soil moisture continued during the entire growth period. In dry and hot summers barley plants were affected by high soil moisture during all the growth periods. However, even in this case the yield diminished less in the critical period.

2. Flooding (soil moisture 120% of total capacity) acts negatively on barley plants during all periods of development. The greatest damage to plants is observed on flooding during the entire period of growth. Their anatomic structure bears a xeromorphic character. Flooding immediately after seeding produces very thin sprouts. Flooding at vernalization stage or in the critical period decreases the yield to a lesser degree than at the light or 3rd stage of development.

3. At the most negative periods of flooding the bound water quantity increases and the free water decreases, and there is a decrease of total hydration at the same time. The pollen viability decreases sharply as a result of disturbances in the normal course of microsporogenesis. Changes in the course of the first reduction and the second divisions of the pollen's maternal cells caused abortive pollen formation.

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PRIMARY INCLUSION OF PHOSPHATES IN ROOT METABOLISM

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The absorption of mineral salts by roots is a physiological process by which practical crop yields can be quite easily influenced. It is therefore natural that plant physiologists have always paid close attention to problems of root nutrition. A well-developed theory of this problem now serves as a dependable basis for agriculture. However, despite great successes attained in the study of root nutrition, the mechanism of ion absorption and primary assimilation still remains insufficiently clarified.

In most recent studies the absorptive activity of roots has been related to their metabolism and particularly to respiration [1, 2, 3, and others]. Nevertheless the close relationship of metabolism to absorption and ion assimilation has thus far been little studied.

To a greater degree than in other cases, this relationship is clarified in the case of the primary assimilation of ammonium ions. Our laboratory in particular guided its studies in this direction and showed that the intermediate products of glycolysis and oxygen respiration forming in the absorptive root zones serve as acceptors through which the primary fixation of NH_2-groups is accomplished [4, 5, 6]. Thus, the relationship of respiration to absorptive activity of roots found a more precise expression in these studies.

In this investigation we undertook to study the primary stages of orthophosphoric acid assimilation by roots. We deemed a study of this essential, both for the direct clarification of the assimilation mechanism of this element important to plant nutrition, and also because the participation of phosphoric acid in cell metabolism is always closely related to the conversion and consumption of energy. Therefore an intimate acquaintance with phosphate conversion in the absorptive zone of roots could also shed light on the energy factor in absorption of other ions.

The study was conducted in 1958 and 1959.

EXPERIMENTAL PROCEDURE

Methodology

Subjects of the study were Mozoleevskaya variety of pumpkin plants cultivated in aqueous cultures on Knop nutrient medium. After 3 weeks the plants were transferred for 4 days to pots with running water to decrease the level of inorganic phosphorus in the roots. The plants treated this way were placed in a solution of NaH_2PO_4 labeled with P^{32}. This solution contained 0.005 mg of phosphorus per ml; its initial radioactivity was 10 mCurie/ml. After a given exposure the plant roots were carefully washed, quickly fixated by liquid nitrogen and analyzed.

To ascertain the nature of compounds in which the phosphorus absorbed by the roots is found, we conducted a fractionation of phosphorus compounds by Ogur's method [7] by successive extractions: a) acid-soluble phosphorus by 10% trichloracetic acid; b) lipoid phosphorus by a 3:1 mixture of ethyl alcohol and ether, and by 1:1 methyl alcohol and chloroform; c) nucleic acid phosphorus by 1 N $HClO_4$ and d) phosphoproteic phosphorus by 2 N NaOH. Acid-soluble phosphorus was in its turn separated into 1) a nucleotide fraction by adsorption on carbon OUD GOST 4453-48 by the Kotel'nikova method [8]; 2) inorganic phosphorus by extraction by the Berenblum method [9]; 3) a fraction of phosphoric esters (hexosephosphates + phosphoglyceric acid) precipitated by barium by the Umbreit method [10]. Moreover, after treating the trichloracetic extract with carbon in many cases we performed additional precipitation from the fraction not precipitated by barium, of phosphoric esters containing nitrogen (phosphorylated choline, phosphorylated ethanolamine and their glycerin esters) by the method of Baer and Kates [11]. The radioactivity of all these fractions was measured. Since the total quantity of absorbed P^{32} in single plants varied, most probably caused by individual characteristics, we recorded the results obtained in percentages of the total labeled phosphorus entering the root tissues. Such a tabulation allowed judging the relative speed of entrance and distribution of labeled phosphorus in different groups of phosphorus compounds.

In working with whole plants the possibility of partial escape of radioactive phosphorus from roots to the above-ground portion of the plant could not be excluded, even though the exposures were very brief. Such an escape could affect the tabulations and conclusions. Therefore, at the start many parallel experiments were conducted with roots on the whole plant and with the roots cut off. It was found that the separation of roots from the plant only decreases the speed of conversions, but that it does not change the essentials of the observed picture. Moreover, it was shown that the escape of P^{32} from the roots of the whole plant in the environments of our experiment is so insignificant that it can be disregarded.

Therefore, in this article we give results obtained in experiments with the whole plant.

TABLE 1. Distribution of P^{32} in Different Groups of Compounds in Roots on Absorption of $NaH_2P^{32}O_4$ (in % of total radioactivity of absorbed phosphate)

Duration of phosphate absorption in minutes	Phosphorus forms					
	acid-soluble			lipoid	nucleic	protein
	inorganic	nucleotide	hexosephosphates*			
	Experiment 1					
1	59.6	12.8	15.1	0.16	5.6	6.7
10	39.9	16.3	20.5	1.3	12.9	9.9
30	41.5	16.7	22.8	0.9	10.7	8.4
60	45.4	14.4	19.3	0.7	12.5	7.7
90	40.2	13.9	24.7	1.1	11.4	8.7
120	39.7	17.1	21.6	1.1	9.2	11.3
	Experiment 2					
1	68.2	14.6	7.1	0.6	5.8	3.7
10	21.4	23.1	14.58	2.7	27.3	10.9
30	26.2	20.1	14.7	3.1	24.1	9.0
60	24.5	19.8	13.0	2.9	28.7	11.1
90	28.7	22.5	14.9	2.4	26.3	15.2
120	29.0	18.9	13.4	3.9	30.2	11.6

*This fraction represents chiefly hexosephosphates, but triosephosphate esters are also included.

Distribution of P^{32} in Different Groups of Compounds with Continuous Absorption of Labeled Phosphate by Roots

It is known that the root system is capable of incorporating its absorbed phosphate into the composition of different organic compounds. In particular, by using P^{32}, intake of phosphorus into protein and nucleic acids was found within the root tissues [12]. However, in most cases the absorptive activity of roots was studied in prolonged experiments, in which the first stages of phosphate metabolism had been passed and overshadowed by secondary conversions. This gave no opportunity for judging either the actual dimensions of phosphate metabolism upon its absorption or of the composition of the initial organic phosphorus products. Moreover, these experiments made it possible to consider that the root system is comparatively inactive toward the absorbed phosphorus, especially since the sap exuded by the roots to the above-ground organs contained chiefly inorganic phosphorus. Meanwhile the unusual speed in the course of many enzymatic plant reactions is frequently evident. It is especially graphically demonstrated in the study of photosynthesis, when the intermediate products can be caught only in the initial seconds after the process begins. Therefore, it would seem that also in the study of phosphorus assimilation by the roots, the mechanism of its assimilation can be elucidated definitely only in short-time experiments.

And in fact, Loughman and Russel [13], who used exposures of one to several minutes, have found that in roots of 10-to 14-day-old barley plants labeled phosphorus is found to be more than 50% incorporated in organic phosphorus compounds within one minute, and first in nucleotides and hexosephosphoric esters.

Similar experiments were also conducted in our laboratory on 25-day-old pumpkin plants. Results agreed closely with those of Loughman and Russel.

According to our findings, some of which are given in Table 1, even after 1 minute of contact with $NaH_2P^{32}O_4$ solution by the pumpkin's root system, a considerable protion of the absorbed phosphorus (up to 40%) is detected in the composition of organic compounds, which indicates a very rapid metabolism (in roots) of the absorbed phosphoric acid. In 10 minutes (sometimes even earlier) after the roots are put in the isotope solution, a more or less constant ratio is established in distribution of P^{32} among the fractions. This ratio is preserved for 2 hours or longer despite the fact that absolute quantities of P^{32}, due to continuing absorption of $NaH_2P^{32}O_4$, uninterruptedly increase in all fractions.

It is seen from Table 1 that the percent of isotopic phosphorus entering different fractions of phosphorus compounds is not the same. The largest portion, at least during the first 2 hours, is found in the acid-soluble fraction, especially in nucleotides and in hexosephosphates. Among the high-molecular phosphorus compounds the nucleic acids and phosphoproteins are enriched most by P^{32}, while phospholipids are very weakly labeled.

The results of experiments 1 and 2 on the whole show a similar picture, even though differences are found in details, probably caused by individuality of plants.

Particularly, the roots in experiment 2 incorporated a larger percentage of inorganic phosphate into the composition of high-molecular organic phosphorus compounds

TABLE 2. P^{32} Distribution in Different Groups of Compounds after a Rapid (30 sec) Introduction into Roots of a Small $NaH_2P^{32}O_4$ Dose (in % of the total radioactivity of the introduced phosphate)

Time after phosphate introduction, minutes	Phosphorus forms						
	acid-soluble				lipoid	nucleic	protein
	mineral	nucleotide	hexose-phosphate	esters not pptd. by Ba			
	Experiment 1						
Initial (30 sec)	70.2	16.5	10.2	0	0	1.7	1.4
1	44.1	32.9	16.1	0.9	0	2.5	2.5
5	27.1	25.0	28.5	1.5	0.4	9.9	7.7
10	22.8	10.2	39.1	1.7	0.39	13.2	11.9
15	32.2	12.5	20.4	3.2	0.7	14.4	12.6
	Experiment 2						
Initial (30 sec)	81.8	8.6	4.4	0	0	2.1	3.2
1	37.6	28.1	27.9	0	0.2	2.8	3.6
5	25.3	7.9	51.7	0.6	0.6	7.9	6.2
10	17.6	5.8	62.8	1.5	0.9	6.4	6.9
15	52.3	7.2	21.7	2.8	1.7	8.1	6.2

than in experiment 1 which, evidently, was related to an enhanced metabolism of nucleic acids. Concordant results were obtained in a number of other experiments. On the whole it should be considered that in environments of continuous absorption of labeled phosphate a similar distribution of P^{32} is typical for pumpkin roots.

At the same time these experiments show that because of a simultaneous (or possibly a rapid succession of) P^{32} incorporation into different phosphorus compounds, observation in greater detail of the fate of absorbed phosphate cannot be made while the supply from external media is continuous. For this, experiments are needed in which the fate of a small dose of P^{32} absorbed by roots in short-time contact with $NaH_2P^{32}O_4$ is followed. Judging by the exceptional speed of phosphate incorporation into organic compounds, the duration of such contact must be curtailed at most to about 30 to 60 seconds. We proceeded with further experiments along these lines.

P^{32} Distribution in Different Groups of Compounds with Short-time Introduction of a Small Dose of $NaH_2P^{32}O_4$ in Roots

In this group of experiments, the pumpkin plants, after being held for a few days in running water, were immersed by their roots for 30 seconds in a phosphate solution labeled with P^{32}. Afterwards the plants were quickly transferred to a medium free of the isotope and at different periods the distribution of phosphorous absorbed by the roots in different groups of substances was determined.

In Table 2, results of two such experiments are shown. It is seen from these that in 30 sec 70-80% of radioactive phosphorus absorbed by roots is still in an inorganic state. However, 20-30% of phosphorus already succeeded during this period in entering organic compounds, chiefly into nucleotides and hexosephosphates.

One minute later we found a further rapid concentration of the absorbed phosphate in nucleotides and other barium-precipitated phosphoric esters which basically comprise glycolysis products, while the phosphoric esters not precipitated by barium (choline and ethanolamine derivatives), which appear to be in the opinion of many authors a transitional form of organic phosphorus in stems [14], are labeled later on. The accumulation of P^{32} in high-molecular phosphorus compounds, noted especially in nucleic acids and phosphoproteins, is considered slower by comparison with nucleotides. Five minutes later, more than 70% of the absorbed inorganic phosphate is incorporated in organic compounds, and 10 minutes later 80% of the phosphate is already metabolized.

The continuing phosphate assimilation at later periods (5, 10 minutes) notwithstanding, the P^{32} content in nucleotides begins to go down rapidly while it increases in other compounds.

The accumulation of P^{32} in the hexosephosphate group proceeds during this period with especial rapidity. This sequence indicates that the introduction of mineral phosphate into the cycle of biochemical conversions in the roots begins with its entry into nucleotides, and probably, primarily into ATP, whence the phosphoric acid residues rich in energy are transmitted chiefly into sugars, the activation of which they bring about, and they are also used up in the synthesis of proteins and nucleic acids.

And finally, 15 minutes after incorporating the radioactive phosphate in the root metabolism, its rapid disappearance is also noted from the hexosephosphates, while simultaneously with it the liberation of inorganic P^{32} occurs.

This last period corresponds, most probably, to the intense glycolysis induced by the preceding period of hexose activation by ATP. As a result, the hexosephos-

phates are converted into substances capable of serving as acceptors of ammonia and other ions, while the phosphate is again liberated in an inorganic form.

As a general outline, this is how the first conversion cycle of phosphate entering roots is accomplished. After this, the second and third cycles can no doubt follow; however, at that time the radioactive phosphate enters very extensively into nucleic acids, proteins, and other cell components, mixes more and more with their own phosphorus reserves and partially is carried by the sap to above-ground organs. Therefore, the picture clearly observed by use of P^{32} in the first minutes loses its clarity later on.

The character of conversion occuring in roots during the initial 10-15 minutes of phosphate absorption is shown graphically in Fig. 1.

The most typical feature of phosphorus metabolism in pumpkin roots is that metabolism of the basic mass of absorbed mineral phosphorus in the initial stages occurs within the acid-soluble fraction, i.e., it is related to the synthesis and utilization of macroergic phosphorus compounds and glycolysis products. These processes proceed with much greater energy than the synthesis of high-molecular phosphorus compounds.

A comparison of our data with results obtained by Loughman and Russel, who studied these phenomena on barley roots, discloses many similarities. This leads to the assumption that formation of macroergic phosphate bonds at the initial stages of phosphorus absorption and the incorporation of energy-enriched phosphate residues into glycolysis products have general importance since they are a part of the root system's energy mechanism. As far as the subsequent stages of phosphate utilization is concerned, they can evidently differ, depending on the characteristics of cell metabolism of one or another plant species.

Sometime after the present study was completed and scheduled for publication, the Finnish investigators Miettinen and Savoioja [15] published an article stating that they successfully separated by paper chromatography the phosphorus compounds forming during the initial 30-60 seconds in pea roots and showing that also in this case the labeled phosphate enters chiefly ATP and ADP as well as uridindiphosphate and some hexosephosphate esters. Thus, these data support the conclusion that primary phosphate assimilation occurs in roots through its incorporation by glycolysis and respiration into ATP, whence the energy-rich phosphoric acid residues are transferred to hexoses, in its turn activating glycolysis and the respiratory cycle.

P^{32} Distribution and Utilization in Roots at Absorption of Other Ions

Lundegardh and Burström [16] established that the absorption by plant cells of mineral nutrient elements is accompanied by intensified respiration. This respiration, named "saline", was investigated in detail in numerous studies [1, 2, 3] which showed a close relationship between cell respiration and ionic uptake. But then, some considered this relationship as only the exchange of respiration carbon dioxide ions for anions of corresponding salts [1]. Sabinin [17] postulated that absorption of substances by the root system occurs at the expense of energy liberated in respiration. However, until recently there was no proof that the energy factor of respiration is necessary to absorption processes. Only recently investigations appeared [18, 19] which have shown that 2,4-dinitrophenol in doses stimulating electron transfer, yet inhibiting synthesis of macroergic phosphates, retards ion absorption. This yielded the first experimental proof indicating the need of energy for ion absorption.

It might be expected that activation of the respiratory process in salt absorption would stimulate processes of phosphate esterification and primarily would hasten synthesis and decomposition of macroergic phosphoric compounds. To clarify this aspect of the problem, important to comprehension of energetics of mineral nutrition, we conducted a series of experiments in which phosphorus metabolism in the root system was compared

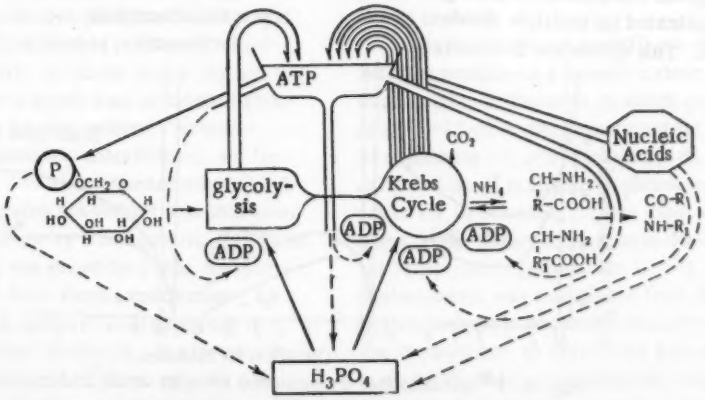

Fig. 1. Primary phosphorus assimilation by roots

TABLE 3. P^{32} Distribution in Different Groups of Compounds following Immersion of Roots in a Solution of Nutrient Salts (in % of total radioactivity of introduced phosphate)

Medium	Duration in min	Phosphorus forms					
		acid-soluble			lipoid	nucleic	protein
		inorganic	nucleotide	hexosephosphate			
Experiment 1							
$NaH_2P^{32}O_4$ initial	1	57.1	23.2	15.7	0.2	1.8	2.0
H_2O	3	43.8	23.4	28.8	0.1	2.2	2.9
Knop $\left(\frac{1}{10}\right)$	3	20.9	32.6	39.5	0	2.9	4.1
H_2O	15	69.3	1.9	18.8	0.7	5.7	3.6
Knop $\left(\frac{1}{10}\right)$	15	23.5	10.3	54.1	0.5	6.3	5.3
Experiment 2							
$NaH_2P^{32}O_4$ initial	1	62.8	16.1	10.5	0.2	5.9	4.4
H_2O	3	46.1	20.1	20.5	0.3	6.9	6.1
Knop $\left(\frac{1}{10}\right)$	3	32.7	28.4	38.9	0.4	6.7	7.1
H_2O	15	60.7	7.2	18.8	0.4	3.7	9.2
Knop $\left(\frac{1}{10}\right)$	15	30.5	11.6	40.7	1.5	4.8	10.9

during absorption of mineral nutrient elements and during a relatively dormant state. For this purpose, after a 60-second contact with a solution of radioactive phosphate, some of the plants were transferred to a 10% Knop nutrient solution, while others (control) were immersed by the roots in running water.

As seen from the data in Table 3, there is greater metabolic activity in the introduced phosphate in plants placed in a solution of nutrient salts than in plants placed in water. This is shown by the fact that in absorbing nutrient mineral elements the rate and magnitude of P^{32} esterification are enhanced. Thus, the metabolism of absorbed phosphorus in experimental plants consisted of 70-80% of the introduced phosphorus within 3 minutes as against 54-57% in the control. In addition, the P^{32} in the salt solution remained in the composition of organic compounds for a longer time, which evidently indicated its multiple turnover in the metabolic process. This difference is especially apparent after 15 minutes, when the larger part of P^{32} was present in organic compounds in the Knop solution, while those in water manifested considerable loss of metabolic phosphate.

The results obtained also show that the intensified esterification process in roots on contact with mineral salts is chiefly related to the metabolic activation of nucleotides and hexosephosphates, i.e., with the energy processes occuring in roots. So far, there are no data by which to judge the concrete mechanism of macroergic phosphate utilization in the process of ion absorption. Nonetheless, it becomes more and more evident that different stages of ion absorption demand energy expenditure and that the processes of oxidative phosphorylation are the necessary sources of this energy.

Figure 2 permits a more graphic view of the energy activation of phosphorus metabolism in roots upon ion absorption

Incidentally, as seen in Table 3, the transfer of

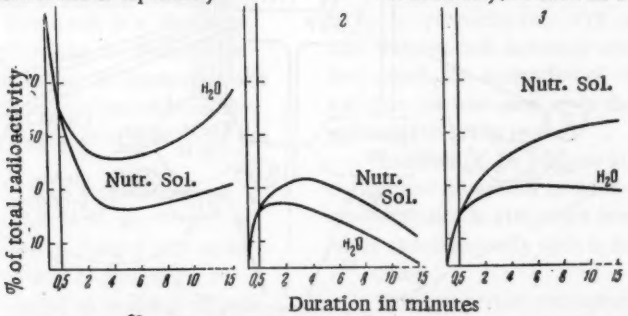

Fig. 2. P^{32} metabolism by pumpkin roots in water and nutrient solution. 1) Inorganic phosphorus, 2) nucleotide phosphorus, 3) hexosephosphate phosphorus.

TABLE 4. Effect of 2,4-Dinitrophenol on the Utilization of Radioactive Phosphorus ($NaH_2P^{32}O_4$) Absorbed by Roots (in % of total radioactivity of introduced phosphate; period of phosphate introduction, 1 minute)

Medium	Duration, minutes	Phosphorus forms					
		acid-soluble			lipoid	nucleic	phospho-protein
		inorganic	nucleo-tide	hexose-phosphate			
		Experiment 1					
H_2O	Init. (1 min)	45.8	14.4	19.3	2.4	12.8	3.3
	10	21.5	18.3	23.9	2.7	21.1	12.5
	15	19.1	10.9	23.8	2.2	28.7	15.3
	20	38.7	10.3	13.5	2.8	27.9	13.5
		Experiment 2					
10^{-4} M Dinitrophenol	Init (1 min)	55.8	15.2	8.9	2.9	13.7	4.5
	10	67.5	2.6	16.6	3.2	6.3	3.8
	15	72.4	0.9	17.5	1.3	2.5	0.9
	20	89.3	0.8	8.1	0.2	0.9	0.7
		Experiment 3					
Before experiment 10^{-4} M Dinitrophenol, Then water	Init. (1 min)	82.3	4.8	12.1	0	0.2	0.6
	10	70.1	9.2	17.3	0.6	0.9	1.9
	15	52.5	15.4	15.9	1.4	10.8	5.0
	20	25.6	15.2	24.0	1.8	22.5	10.9
		Experiment 4					
H_2O Knop + Dinitrophenol 10^{-4} M	Init. (1 min)	69.1	12.6	10.3	0	7.19	0.81
	(5)	37.3	10.1	37.5	1.4	9.6	4.1
	(5)	52.1	1.8	31.2	1.6	8.9	4.4

plants to a nutrient solution not only activates glycolysis and formation of macroergic phosphoric compounds, but in many cases it also intensifies the synthesis of phosphoproteins and nucleic acids.

P^{32} Distribution and Utilization in Roots when Oxidative Phosphorylation is Poisoned by 2,4-Dinitrophenol

For further clarification of the nature of energy in root absorption activity we tried application of specific poisons paralyzing one or another part of the metabolism.

In the present study we used 2,4-dinitrophenol as an inhibitor poisoning oxidative phosphorylation, even though it is not strictly specific (as, incidently, most respiratory poisons are not). As in the preceding experiments, we introduced a small dose of labeled phosphate into pumpkin roots for one minute. However, after the phosphate was partially metabolized, we immersed the roots in a 10^{-4} M dinitrophenol solution. A second group of plants serving as a control was immersed in pure water after treatment by a radioactive phosphate.

The results obtained are shown in Table 4(experiments 1 and 2). As seen from these experiments, even after 10 minutes the action of dinitrophenol begins to manifest itself rather distinctly. It expresses itself in a rapid disappearance of P^{32} from organic compounds and in a corresponding accumulation of inorganic phosphorus. This process continued in the given experiment during the entire period of observation and culminated in the liberation of almost 90% of previously assimilated phosphate.

It is seen from the experiments that the poisoning of the oxidative phosphorylation hastens decomposition of all organic phosphorus compounds in roots. However, processes of primary esterification proved to be most sensitive to this treatment since the P^{32} reserve in the nucleotides are exhausted first as a result of dinitrophenol activity. This can be explained by the fact that the delay of ATP synthesis by the inhibitor's action leads to a rapid consumption of previously formed macroergic phosphates and to a liberation of inorganic phosphorus bound in them.

The dinitrophenol activity on phosphorus metabolism is reversible to a certain extent. This is seen from experiment 3 (Table 4) in which pumpkin plants were immersed before the experiment for 30 minutes in 10^{-4} M dinitrophenol, and afterward, on receiving a one-minute dose of radioactive phosphorus, were kept in water for 20 minutes. In this case, as seen from experiment 3, the absorbed phosphate dose initially metabolized very poorly (altogether 17.7%). However, as the dinitrophenol was washed out from the roots, the radioactive phosphate became more and more involved in the metabolism, so that at the end of the experiment, nearly 75% of labeled phosphate was found in the composition of nucleotides, hexosephosphates and other organic compounds.

Earlier it was pointed out (see Table 3) that on contact of roots with Knop solution the incorporation of inorganic phosphate in the composition of nucleotides and hexosephosphoric esters is increased, which indicates an internal manifestation of the so-called saline respiration. In presence of an inhibitor (dinitrophenol) roots lose the capacity of oxidative phosphorylation and therefore on immersion into Knop solution, they probably will not be in a condition to activate their energy metabolism.

This supposition was confirmed in experiment 4 (Table 4) in which the roots, after introduction of radioactive phosphorus for 1 minute, were immersed in a Knop solution containing 10^{-4} M dinitrophenol. In this case, differentiating from experiments conducted on pure Knop solutions (see Table 3), the roots immersed in a mixture of Knop solution and dinitrophenol not only did not increase their energy exchange but on the contrary, they made considerably less use of inorganic phosphate than in water alone. As should have been expected in such cases, the intake of P^{32} in nucleotides was found to be especially strongly inhibited.

At the same time, as frequently noted in the literature, roots poisoned by 2,4-dinitrophenol lose the capacity to absorb ions [13]. This once more indicates the close relationship of the roots' absorptive activity to their energy exchange, in which the leading role belongs to macroergic phosphorus compounds (ATP and others).

Further study of the problem will aid in greater comprehension of this relationship.

SUMMARY

Mineral phosphorous ($NaH_2P^{32}O_4$) entering the roots of 25-day-old pumpkin plants rapidly becomes involved in metabolic processes and after 30-60 seconds, 30% of it is found in organic compounds. Later on (in 3-5 minutes) about 70% of the absorbed phosphate is incorporated in organic compounds (Table 1).

Inclusion of absorbed phosphorus in metabolic processes begins with its entering nucleotides (ATP, etc.) with subsequent transfer of energy-rich phosphoric acid residues to other organic compounds and firstly to sugars. Accumulation of P^{32} in phosphoproteins and nucleic acids takes place at a slower rate (Table 2).

Phosphorus metabolism is intensified when the root system comes in contact with mineral salts. In this case the amount of P^{32} in nucleotides and hexosephosphoric esters increases. This indicates an enhancement of energy exchange in the roots during this period (Table 3 and Fig. 2).

2,4-Dinitrophenol, which is known to impede absorption of ions, sharply inhibits the formation of nucleotides in roots. This leads to rapid consumption of earlier produced nucleotides and hexosephosphates and to the accumulation of inorganic phosphorus. Phosphorus metabolism of such roots is not intensified when they come into contact with the mineral nutrient solution (Table 4).

The foregoing leads one to the conclusion that the absorbing power of roots is closely related to energy exchange in the absorbing cells.

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THE ROLE OF LEAVES IN THE VERNALIZATION OF WINTER CEREALS AND BIENNIALS

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It is well known that winter cereals and certain biennials (wheat, turnip, etc.), are vernalized with equal success as germinated seeds, the endosperm of which contains reserve nutrients, or as green plants. Vernalization of many biennials is not completed in the seed stage but in the green plant.

Because of this, many workers have concluded that vernalization in these species bears a relationship to plant age. Mikhailov [1] and Chesnokov [2] have shown that cabbage and carrot in hot beds can be vernalized only at two months of age. Razumov [3] has es tablished that in carrot, vernalization is begun in the seed and completed in the green plant. On this basis, and in the light of findings of other workers, he concludes that plant age is of no significance for the commencement of vernalization.

It should be pointed out, however, that the role of individual organs (leaves, storage roots, etc.), has not been studied at all. The role of leaves in the initiation and differentiation of floral buds, processes dependent on the photoperiodic regime, has, on the other hand, been widely discussed, and the results of studies on this point have been put into practice. An investigation of the role of leaves in vernalization, in addition to being of purely theoretical interest, might be of practical value in plant cultivation. In view of this, we performed appropriate investigations.

Experiments on the vernalization of winter wheat and certain biennials, and also of grafts of biennials on spring annuals and on biennial seed plants were carried out. In the case of seedling vernalization, plants of various ages with a limited number of leaves and without them were used. In the case of graft vernalization, leaves were left at selected nodes on the stocks. Scion leaves were either removed or left intact. In order to evaluate the thoroughness of vernalization, plants were subsequently grown at the day length which would ensure normal flowering. Experiments were performed in greenhouses and chambers of the phytotron at the K. A. Timiryazev Institute of Plant Physiology, USSR Academy of Sciences, Moscow. Plants were grown in pots and flats, the experiments being replicated four times. Ten winter wheat and five biennial plants were grown in each pot. Heading in wheat was considered normal if more than 90% of the plants were heading, and sprouting of biennials if more than 60% of the individuals produced shoots.

Experiments With Winter Wheat, Wheat-Agropyron Hybrid No. 599

Our experiments showed that rate of vernalization depends on plant vigor and leaf surface area: The higher it is, the more rapidly vernalization proceeds (Table 1).

When plants were vernalized just after the disappearance of endosperm, when they had produced one or two immature leaves but had not produced tillers with their nutrient reserves, vernalization and floral induction were slow and occurred only on a long day (treatment 2, Fig. 1).

Plants with tillers were vernalized more rapidly, and headed earlier than those vernalized in the seed stage. This emphasizes the more active role of leaves as compared with endosperm in the vernalization process (treatment 5, Fig. 2).

If a single vigorous leaf and the tillers are left intact, vernalization proceeds normally. Even in the absence of leaves, but in the presence of tillers containing nutrient reserves, vernalization can occur in the dark, and at almost the normal rate, as seen by the number of days to differentiation of the terminal meristems (treatment 6, 7) and to heading (Fig. 3). Vernalization without leaves in the dark is retarded because of a deficiency of nutrients in the tillers. In the light, vernalization is a little more rapid than in the dark (treatments 5-7). In well-developed plants with tillers, floral induction proceeds normally on a short day as well (treatment 8, Fig. 4).

Thus, vernalization of plants which have used up their endosperm and have produced one or two immature leaves, but no tillers, is very slow. In plants with tillers and without leaves, vernalization proceeds normally even in darkness, and floral induction is accomplished even on a short day, which indicates the importance of accumulated nutrients. As has been shown experimentally, if endosperm is removed from wheat seeds or if storage organs are removed from biennials, vernalization and differentiation of isolated embryos or buds fail to occur because of a lack of nutrient reserves [4-8]; it only occurs with the appearance of leaves or under conditions in which sugars are artificially supplied.

TABLE 1. The Effect of Leaf Area During Vernalization on Development of Winter Wheat (vernalization 40 days at 2-5°, then long days)

Treatment No.	Plant condition prior to vernalization	No. of leaves	Leaf area prior to vernalization, cm^2	No. of days after vernalization to:	
				differentiation of growing points	heading
1	Germinated seeds (embryo with endosperm)	None	-	12-15	59-60
2	Fifteen days after sprouting (after disappearance of endosperm, no tillers)	1	10-12	30-40	72-74
3	The same	2	20-23	28-35	66-67
4	Thirty days after sprouting (with tillers)	1	20-25	8-10	55-56
5	The same	4-5	90-100	5-7	51-53
6	The same (vernalization in the light)	None	-	8-10	58-59
7	The same (vernalization in the dark)	None	-	10-12	61-63
8	The same (after vernalization on a short-day – SD)	4-5	90-100	5-6	50-52

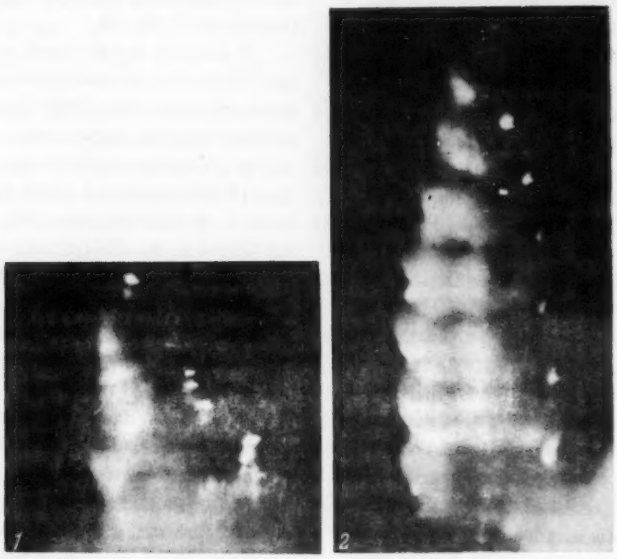

Fig. 1. The effect of day length on the differentiation of growing points in winter wheat, Wheat-Agropyron hybrid No. 599. Plants vernalized with one or two leaves, without tillers. 1) After vernalization on a short day; 2) after vernalization on a long day.

Fig. 2. Effect of length of vernalization on seeds and on plants of winter wheat. 1) Seeds vernalized 30 days, long day (LD) given from sprouting (from April 7) – no heading; 2) plants vernalized 30 days, then given LD (from April 4) – heading after July 5; 3) seeds vernalized 40 days, LD from sprouting (from April 7) – no heading; 4) plants vernalized 40 days, then given LD (from April 13) – heading June 24; 5) plants vernalized 50 days, then given LD (from April 25) – heading June 15.

Fig. 3. The effect of tillers on vernalization of winter wheat. Vernalization 40 days at 2-5°. 1) Vernalization with all leaves and with tillers in the light – heading August 1-5; 2) vernalization with all leaves and with tillers, but in the dark – heading August 14-16; 3) vernalization with tillers and in the dark, but without leaves – heading August 23-25.

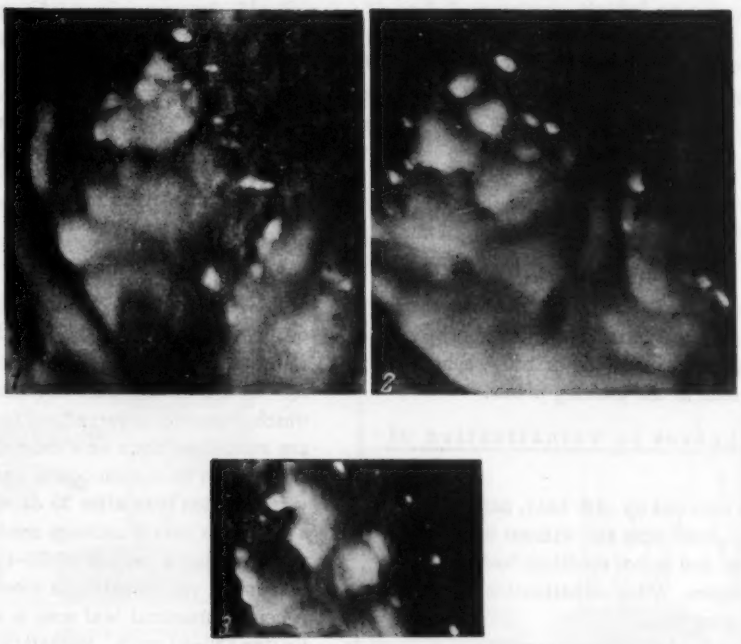

Fig. 4. The effect of day length on development of vernalized winter wheat plants, vernalization 40 days at 3-5° (head apices). 1) After vernalization, long day (LD) continuously; 2) after vernalization, short day (SD) continuously; 3) after vernalization, 10 days in the dark, then LD.

TABLE 2. The Effect of Leaf Area during Vernalization on the Development of Biennial Seedlings of of Various Ages (based on the area of one side of a leaf)

Plant age before vernalization			Duration of vernalization (days)	Shoot formation (days after vernalization)
from germination (days)	number of leaves	leaf area, cm^2		
		Bordeau table beet		
30	3-4	15-18	45	20-30
48	4-5	30-40	45	16-25
92	None	-	40	No shoots formed
		P-06 sugar beet		
30	3-4	25-30	30	15-20
30	3-4	25-30	45	8-12
40	4-5	30-40	35*	40-45
40	4-5	30-40	50*	22-25
		Nomer pervyi cabbage		
30	2-3	18-20	65	No shoots formed
35	3-4	35-40	70	65-70
42	4-5	50-60	65	38-39
53	6-7	90-100	65	18-19
94	None	-	65	No shoots formed
175	More than 12-15	More than 200	30	No shoots formed
175	More than 12-15	More than 200	50	37-38
		Nant-skaya carrot		
27	3	5-10	70	No shoots formed
37	4-5	25-30	90	30-35
80	None	-	80	No shoots formed

*Vernalization on a short day.

This is an indication that qualitative developmental changes occur first of all in the metabolism of storage and photosynthesizing organs, and that they subsequently become localized in the growing points.

The Role of Leaves in Vernalization of Biennials

Experiments were set up with beet, carrot, and cabbage seedlings, both with and without leaves. At this time, the beet and carrot seedlings had not as yet formed storage organs. After vernalization the plants were grown on a long day.

According to our observations, vernalization depends on leaf area and on plant age (Table 2); as they are increased, the period required for vernalization is shortened and shoot formation occurs at an earlier period.

Forty-eight-day-old Bordeau beet seedlings, which had a leaf area of 30-40 cm^2, formed shoots a little earlier than did 30-day-old seedlings with a leaf area of 15-18 cm^2 (Table 2, treatments 1, 2). Plants without leaves, in spite of the fact that they were older, were not vernalized and did not form shoots (treatment 3). Similar results were obtained with P-06 sugar beet, which is normally vernalized in 30 days. Older plants are vernalized even on a short day; under these conditions, shoot formation occurs earlier after 50 days' vernalization than after 35 days' vernalization.

Nomer pervyi cabbage seedlings undergo normal vernalization at an age of 35-42 days, in the four-five leaf stage; vernalization is more rapid at later stages. A certain minimal leaf area is a prerequisite of vernalization (30-40 cm^2). Vernalization is more rapid in plants with vigorous and well-developed leaves. At the same time, vernalization time and time to differentiation of growing points in 175-day-old seedlings approximates that of seed-bearing plants with storage organs (heads). The important factor is not, therefore, calendar age, but plant vigor, as expressed by leaf number and leaf area.

It must be noted that vernalization of biennials with leaves in the light was more rapid than on a short day or in darkness (as with winter cereals). According to our findings, for example, 50-day-old seedlings of the early-maturing Nomer pervyi cabbage produced shoots 50-52 days after a 70-day vernalization in the dark, while after vernalization under continuous illumination shoot formation occurred in 20-25 days.

Observations have shown that the longer the vernalization period, the earlier shoot formation occurs. Many workers have observed similar behavior in other plants. Bordeau beet seedlings 30 days of age with a leaf area of 15-18 cm^2 are vernalized after 20 days. With shorter vernalization periods, shoot formation was retarded. Nant-skaya carrot seedlings 37 days old with four to five leaves required 90 days for vernalization. Longer periods did not hasten shoot production. After long vernalization periods, plants of some species produce shoots even on a short day.

Interesting results were obtained with vernalization of seedlings without leaves. To ensure success, plants were selected for this experiment which were twice as old as in other experiments, vernalization at this age usually being rapid. When, however, such plants were transferred to the greenhouse after vernalization, they failed to produce shoots or to flower, even though the leaves at the shoot apex grew rapidly and assumed an almost normal habit after a month (Fig. 5). In carrot and beet plants lacking storage organs containing nutrient reserves, the leaves grew feebly and many plants soon died. The remaining plants, however, also failed to produce shoots; therefore, without leaves they had not been vernalized.

As is now known, in cabbage the growing points are differentiated at the low temperature prevailing during vernalization [8, 9]. Therefore, removal of leaves during floral induction of seedlings (after vernalization) did not retard differentiation. Floral induction, however, did not occur, and floral initiation was prevented by a deficiency of nutrients. Under these conditions, the seedlings died.

Removal of leaves from cabbage and onion plants in the seed-bearing stage (Fig. 6) did not inhibit shoot production, since differentiation had already occurred, and further growth was maintained at the expense of large nutrient reserves; if the bulb scales of onion were removed, growth of the shoots was completely inhibited. Removal of leaves from vernalized seed plants of root crops at a time of leaf growth, in the floral induction stage, completely suppressed bud differentiation, shoot production, and shoot growth [10]; in the presence of leaves, however, shoots appeared even in the dark, although no inflorescences were formed; without leaves, there was no shoot formation in the dark. When this does occur, it indicates that floral induction has occurred, and the inhibition of flowering is related to other requirements at the third and fourth stages of development.

Vernalization of biennial seedlings in the absence of leaves and of storage organs (storage roots, heads, etc.) does not occur, therefore, at any age. A sufficiently developed assimilation apparatus (area greater than 20-30 cm^2) is necessary. Neither does floral induction of vernalized seedlings lacking leaves occur. If vigorous storage organs are present (in seed plants), then both vernalization and floral induction occur and the plant produces shoots even in darkness. Here the age of the storage organs is not critical.

When unvernalized biennial seedlings are grafted onto flowering spring annuals, the scions are vernalized even at high temperatures, and floral induction is in some cases accomplished even on a short day. We have

Fig. 5. The effect of leaves on vernalization of Nomer pervyi cabbage seedlings. Age 53 days, vernalization at 3-5° for 70 days, long day from December 10. 1) Vernalization without leaves – no shoots formed; 2) vernalization with leaves – shoot formation January 17-19, flowering February 18-20.

Fig. 6. The effect of leaves on shoot formation in vernalized Bessonov onion 1) Without leaves and scales-no shoots formed; 2) without leaves, but with scales, shoots formed; 3) with leaves and scales- shoot formation and growth normal.

established that grafted seedlings are vernalized and produce shoots as readily as ungrafted ones. Leaves must be removed from cabbage scions under these conditions, but in carrot and beet this is not necessary. The role of leaves of stocks also varies: On mustard and rape stocks grafted to cabbage, no fewer than one or two leaves must be left intact. These leaves must be the upper leaves; if the lower two leaves are left, the cabbage scion fails to form shoots (Fig. 7), this being due to a flow of nutrients from the scion predominantly into the root system [11].

It is not absolutely necessary to retain leaves on a dill stock grafted to carrot, since the scion produces shoots even on a completely defoliated green stock; this is an indication of the importance of the latter. If the stems are shaded, the scion does not produce shoots.

It should also be pointed out that seedlings of root crops grafted onto flowering plants of the same species (carrot onto carrot, beet onto beet) are certain to undergo vernalization, floral induction, and shoot formation either with or without leaves, which indicates the importance of the old storage roots which nourish the scions through their stems.

Thus, the role of leaves in the development and flowering of plants is strikingly demonstrated in grafts of biennial seedlings onto annuals or onto biennials of the same species in the reproductive stage. Under these conditions, the leaves, stems, and storage organs of the stock play an important role; as a rule, leaves of the scion do not hinder floral induction or shoot formation, with the exception of cabbage. When cabbage is grafted onto cabbage in the reproductive stage, it does not produce shoots without supplementary vernalization regardless of whether the scion has leaves or lacks them.

Fig. 7. The effect of position of the leaf retained on a rape stock on the development of a Nomer pervyi cabbage scion. 1) One upper leaf left on the stock – shoot production July 18; 2) lower leaf retained – no shoot production.

In conclusion it should be pointed out that in winter cereals and biennials leaves play a most important role in vernalization. They may be replaced only by organs with sufficient amounts of accumulated nutrient reserves (endosperm of seeds, tillers of cereal grasses, storage roots, cabbage heads, onion bulbs, etc.). However, leaves ensure vernalization only if a certain minimal surface area is present; for beet this is 15-20 cm^2, and for cabbage and carrot it is more than 30-40 cm^2. Removal of leaves (and also of storage organs) inhibits the vernalization process. Floral induction and bud differentiation in winter cereals and biennials also fails to occur in the absence of leaves.

We have also shown that in annuals the greater the leaf area, the more rapid is differentiation [12].

On the basis of these and other studies, it may be concluded that in all cases leaves ensure the completion of vernalization, while only in certain biennials, mainly earlier varieties with short vernalization stages, is this process completed in the seed stage. For example, in seeds of carrot and late varieties of cabbage, vernalization is only begun, being completed in post-germination stages. Winter wheat, radish, and other crops are vernalized more rapidly and produce shoots sooner when low temperatures are applied to plants rather than to seeds. Therefore, leaves are more active than are seeds.

However, biennial seedlings cannot be vernalized equally well at every age. Ease of vernalization depends not on total plant age, but primarily on area of the assimilation surface responsible for the accumulation of a sufficient amount of nutrient materials, which are concentrated in the growing points. At early stages, when the leaf is being formed, it uses up the assimilates in its own growth. Only when its growth slackens off do assimilates flow to other organs in greater amounts. It is only in this sense that leaf age is a factor in vernalization.

V. I. Razumov [13, 14], basing his conclusions on our experiments and those of other workers touching on ease of vernalization in seeds, onion bulbs, and storage roots as related to their maturity, also believes that this depends on nutrient reserves; the more abundant they are, the more rapid is vernalization.

We have come to the conclusion that processes involving sequential stages (including vernalization) are related to metabolic events and at first occur in storage and photosynthesizing organs, being subsequently localized in the growing points. A most important role is played by a nutrient complex, the constituents of which vary widely as to quantity and quality. Stimulators and inhibitors may only accelerate or retard a particular metabolic process, but are unable to replace these nutrients.

SUMMARY

Seedlings of biennials in which the vernalization stage terminates when the plant is green require a certain minimal leaf surface area in order to pass through the vernalization stage. Vernalization does not occur in plants deprived of leaves. In those plants in which vernalization can successfully occur in germinating seeds as well as in the green plant, the endosperm with its reserve of nutrient substances, or a certain leaf area, is required. Thus, in biennial plants, the area should be not less than 20-30 cm^2, and winter plants, moreover, should have well-developed tillers. Vernalization proceeds at a slow rate during the sprouting period, when the endosperm is exhausted, and the leaves have not yet developed.

Vernalization can take place in biennial seed plants deprived of leaves, but only in the presence of reserve organs (roots, bulbs). In the absence of reserve organs and old leaves, vernalization of buds can take place only if new leaves appear. Bud differentiation and shoot formation can occur in some plants (cabbage, onion etc.) without leaves, whereas in other plants leaves are required (e.g., in root crops), the presence of reserve organs being an indispensable condition for each group.

If biennial seedlings are grafted onto annual plants or their own seed plants, vernalization, floral induction, and also bud differentiation of the biennial plants proceeds under the influence of the leaves, stems and reserve organs of the stock. As a rule, leaves left on the plant do not have any effect on flowering. An exception is cabbage; when its seedlings are grafted onto annual plants, leaves of the scion should be removed. If the plant is grafted onto its own seed plant the graft should first be vernalized. If this is not done, shooting out will not occur.

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A STUDY OF PHOTOREDUCTION IN SCENEDESMUS OBLIQUUS USING C^{14} AND O^{18}

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It is well known that certain algae can reduce carbon dioxide in the light under aerobic (photosynthesis) and anaerobic (photoreduction) conditions [1]. The elucidation of the mechanism of photoreduction in algae is of great value in the study of the chemistry and evolution of photosynthesis in plants and in bacteria. At the present time, the similarity in carbon dioxide reduction by algae during photosynthesis and photoreduction, as judged by the distribution of C^{14} in initial and final products [2-4], appears to be of interest. The question of a similarity in photochemical stages of photoreduction and photosynthesis is just beginning to be investigated, however. Up till now there are only data indicating that quantum yield and carbon dioxide absorption are the same for the two processes, as indicated by experiments with interrupted light, which provide information relating to the photochemical stage [5, 6]. The hydrogen donor for carbon dioxide reduction in adapted algae during photoreduction, as well as that for photosynthesis in bacteria, is unknown [6, 7].

There are two hypotheses: Either it is molecular hydrogen (and also hydrogen of supplementary donors) or the hydrogen of water, as during photosynthesis in plants [1]. The nature of the hydrogen donor involved in photoreduction by cells of Scenedesmus obliquus is the subject of this paper.

Earlier, it was found, using O^{18}, that the oxygen of carbon dioxide and, to a lesser degree, that of water, participates in the synthesis of organic compounds during photosynthesis and photoreduction in algae [4, 8]. Using this information, we decided to attempt to clarify the existence of photolysis of water in photoreduction by the following method: We studied simultaneously the isotopic composition of oxygen and the radioactivity of carbon in the algal cells during photosynthesis and photoreduction using $C^{14}O_2$ and H_2O^{18}. A comparison of results obtained for these processes should give information as to the isotopic composition of the oxygen of the organic compounds synthesized and as to the oxidative metabolism of the cells.

In addition, the effect of time of year on the isotopic composition of oxygen and on carbon dioxide incorporation by the algae was studied.

Experiments were performed in Barcroft manometers for one hour at 22°. For the photosynthesis experiments, the gas phase consisted of air and 4% radioactive carbon dioxide (specific activity – 0.7 microcuries per gram). The algal cells were kept in pH ~8 phosphate buffer ($^1/_{15}$ M), which was prepared using water enriched with O^{18}; enrichment level was 0.75%. In the photoreduction experiments, the cells were adapted in a hydrogen atmosphere for three hours, then given radioactive carbon dioxide and phosphate buffer enriched with O^{18}, and exposed to light. In order to separate photosynthesis and photoreduction, hydroxylamine ($1 \cdot 10^{-2}$ M) was employed as usual. Radioactivity of the dried cells was determined with a Geiger-Mueller counter. Carbon dioxide absorbed was calculated on the basis of 100 mg absolutely dry cells (or in % C^*O_2 incorporated per unit cell weight). The oxygen of the cells was obtained in the form of water according to the method of Ter-Meilen, a modification of that proposed by Teis [9]. This method consists of a catalytic hydrogenation of the organic material to water. The isotopic composition of the oxygen in the water thus obtained was determined by the mass spectrometer according to the isotopic composition of the oxygen of the carbon dioxide in equilibrium with the water. Isotopic composition of the oxygen in the algal cells was expressed as ΔO^{18} (%), which represents the difference in concentration of heavy oxygen from water obtained in the experiment, which was in turn obtained from oxygen

TABLE 1. Isotopic Composition of Oxygen and Carbon Dioxide Incorporation of Scenedesmus obliquus Cells at Various Times of the Year (light intensity 3000 lux)

Date of expt.	ΔO^{18}, %	C^*O_2 incorp. per unit cell wt, %	ΔO^{18}, % / % C^*O_2
	Photosynthesis		
1/15	0.011	1.159	0.009
3/15	0.018	1.301	0.014
5/15	0.022	1.622	0.014
	Photoreduction†		
1/15	0.009	0.691	0.013
3/15	0.016	0.798	0.020
5/15	0.020	0.983	0.020

† Hydroxylamine used.

of the algal cells, and natural (river) water (0.2 atom percent). Results of these experiments are presented in Tables 1 and 2 (average values for four or five experiments).

TABLE 2. Isotopic Composition of Oxygen and Carbon Dioxide Incorporation of Scenedesmus obliquus Cells† (light intensity 2000 lux)

Process of carbon dioxide incorp.	Presence of hydroxylamine	ΔO^{18}, %	% C^*O_2 incorp. / unit cell wt.	$\frac{\Delta O^{18}, \%}{\% C^*O_2}$
Photosynthesis	—	0.009	0.890	0.010
Photosynthesis	+	0.003	0.020	—
Photoreduction	+	0.007	0.480	0.015
Dark control	—	0.004	0.030	—

†Experiments performed in December

The study of carbon dioxide incorporation at various times of the year showed that algae grown in the laboratory under strictly constant conditions have a higher over-all metabolic activity in the spring months than in the winter months. This is evidently due to the onset of a dormant stage in the winter months during cultivation under artificial conditions. The time of year is reflected in rate of cell division [10], absorption of labeled carbon dioxide, and O^{18} enrichment of cells during photosynthesis and photoreduction.

During respiration in the dark, there is also an accumulation of O^{18} by the cells, but it is considerably smaller than in the light [4, 11].

It should further be pointed out that there is a certain analogy between photosynthesis and photoreduction with respect to carbon dioxide incorporation and O^{18} enrichment of cells which attests to the participation of the oxygen of water in the synthesis of organic materials associated with these processes. If, however, the ratio of O^{18} enrichment to amount of carbon dioxide absorbed (ΔO^{18}, %/% C^*O_2) is calculated, it is found to be higher for photoreduction than for photosynthesis. This is indicative that O^{18} enrichment of materials synthesized is greater during photoreduction than during photosynthesis, and also that the patterns of oxidative metabolism a associated with the two processes are distinct.

The greater O^{18} enrichment of synthetates during photoreduction may either be due to a photolysis of water in the photochemical stage, or it may be the result of a heightened utilization of water containing O^{18} by metabolic reactions. The latter alternative seems to us an improbable one in view of the smaller amounts of carbon dioxide absorbed during photoreduction and the smaller changes in the organic phosphorus fraction. The first alternative appears more convincing for this reason [4, 12].

The following mechanism for photoreduction in algae may be proposed on the basis of these data. Photolysis of water occurs in the photochemical stage of both photoreduction and photosynthesis, and its hydrogen is utilized in carbon dioxide reduction. During photosynthesis, its oxygen is released through intermediate peroxides, while during photoreduction these peroxides are reduced by gaseous hydrogen by means of secondary enzymatic reactions, the hydrogen being activated by hydrogenase. The water formed in this reduction participates in its turn in the synthetic reactions of photoreduction. This mechanism accounts for our observation that during photoreduction there is actually greater O^{18} enrichment of materials synthesized than during photosynthesis. The only difference between these two processes is that during photoreduction oxygen is not evolved.

In conclusion, the author expresses his deep gratitude to R. V. Teis and V. M. Kutyurin for valuable advice in the course of this investigation.

SUMMARY

1. Using C^{14} and O^{18}, information was obtained relating to the photochemical stage of carbon dioxide reduction under aerobic (photosynthesis) and anaerobic (photoreduction) conditions in Scenedesmus obliquus.

2. A comparison of the isotopic composition of oxygen in the algal cells during photoreduction and photosynthesis revealed that O^{18} enrichment of the cells is almost the same for both processes. In the light, O^{18} content was higher than in the dark.

3. A comparison of photoreduction and photosynthesis with respect to the ratio of O^{18} enrichment to C^{14} incorporation showed, however, that the oxidative metabolism of the cells differs in the two cases.

4. Organic material synthesized during photoreduction was more enriched with O^{18} than that synthesized during photosynthesis. This indicates that in the photochemical reaction of photoreduction, photolysis of water is possible, just as in photosynthesis.

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AN INVESTIGATION OF THE OPTICAL PROPERTIES OF LEAVES OF WOODY PLANTS USING THE SF-4 SPECTROPHOTOMETER

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The optical properties of leaves have long interested investigators studying the light physiology of the leaf and the processes of photosynthesis. During the last two decades a considerable body of information has been accumulated [1-14] which yields a coherent view of the optical properties of white, green, and anthocyanin-containing leaves functioning under various conditions of environment. At present, these studies are attracting a great amount of attention.

Trees and shrubs of the central zone of the USSR, which comprise a group heterogeneous in a systematic and an ecological sense, are nevertheless characterized by certain common features. Among these is a vigorous leaf complement with an enormous capacity for withdrawing water upward from the soil and with a high light-energy requirement; plants with leaves of this type belong on the whole to the mesophyte group, but are somewhat xerophytic in leaf structure.

Since published information on the optical properties of leaves of trees and shrubs is scanty [1, 2], we have undertaken an investigation of this subject with plants of the central zone of the European sector of the USSR.

METHODS

An analysis of methods of study of optical properties of leaves revealed that at present there is no standard equipment for the performance of such studies.

The method proposed by us earlier [11] cannot claim wide adoption because of the necessity of obtaining amplifiers of various sensitivities, as determined by the intensity of the monochromatic light, the light dispersion, etc.

In this communication a more refined method is described, which is based on the widely used SF-4 spectrophotometer [15].

The SF-4 spectrophotometer is often used as a monochromator; in this case, the photocell housing is removed and the light emerges from the cuvette compartment in the form of a monochromatic beam.

The attachment we are proposing is schematically illustrated in Fig. 1.

If the photocell housing is removed, the attachment with the optical sphere (d = 130 mm), can be slid into its place along guide rails. In principal the sphere is the same as one described previously [11].

The slit plate (Fig. 1, 4 and 2, A) has an aperture (d = 5 mm), the center of which is aligned with the center of the exit opening of the cuvette compartment.

The monochromatic beam passes through the opening of the slit plate 4 (Fig. 1), the barrel 5, and the slide adapter 2, and enters the sphere. When the exit opening of the sphere is closed by a reflector, the light remains inside. The sphere is supplied with adapters 2 and 12 for slides 24, 25, the principles of construction and the significance of which have been previously described [11]. The barrel (d = 20 mm) fits into a holder 3, which is joined to the left adapter 2 of the sphere, and is held in place by a screw 6. When the photocell housing has been replaced by the attachment together with the slit plate, the sphere support 17 is lowered. With

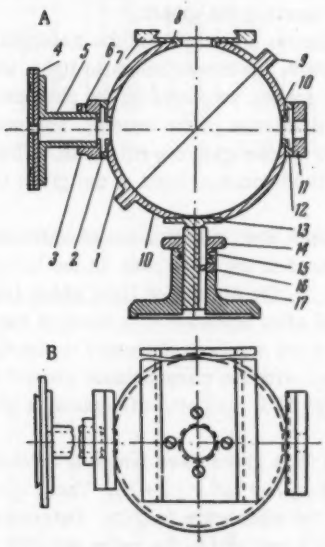

Fig. 1. Schematic diagram of the attachment to the spectrophotometer. (Explanation in text). A) Lateral view; B) top view.

the jack screw 14, it is possible to elevate the core of the support 13 to the surface of the sphere, thereby shifting the center of gravity of the attachment from the slit plate to the support 17. On the upper side of the barrel is a bubble level which, together with the jack screw, 14 facilitates centering of the entrance opening of the slit plate and the exit opening of the sphere to obtain a horizontal beam. A second slit plate 7 with an opening 30 mm in diameter is attached to the upper side of the sphere (Fig. 1 and Fig. 2, B); the photocell housing can be easily slid into position so that its window, which is supplied with a shutter, is exactly aligned with the upper opening of the sphere. During measurements of the optical properties of leaves in the 420-700 mμ range, light from the sphere impinges on a blue-sensitive photocell (220-650 mμ) or a red-sensitive photocell (600-1100 mμ).

The attachment is made of duraluminum and an organic vinyl plastic. The inner surface of the sphere is coated with a reflecting paint.

Transmittance T and reflectance R were recorded with the potentiometer and amplifier of the spectrophotometer itself.

Optical properties of leaves were determined in the following manner. Slides with leaves held in place by clamps were placed in the slide adapters. At first the right-hand slide was fixed in such a position that the exit opening of the sphere was blocked, and light was prevented from entering the sphere by closing the shutter in the cuvette compartment; with the photocell open, the dark current control was rotated to zero the milliampere meter needle. The transmittance control was set at 0% transmittance to compensate for possible scattered light entering the sphere.

After the shutter is opened and the transmittance control set at 100%, the monochromatic light, which is scattered in the sphere, impinges on the photocell, as indicated by a deflection of the needle. The needle is set back to zero by changing the slit width. This position corresponds to 100% incident light at the given wave length.

When the leaf, the transmittance of which is to be measured, is placed in the light path in the left slide adapter (Fig. 1, 2), the amount of light which impinges on the photocell after repeated reflections in the sphere is decreased and the needle is deflected to the right; it is rotated to zero with the transmittance control and the transmittance (in %) is read off; reflectance is then determined.

Absorption for a given wave length is calculated from the equation, $A = 100 - (T + R)$. These operations are carried out for other wave lengths. Determination of absorption by a leaf within the range 400-700 mμ takes 7-10 minutes, and does not require further treatment. A few minutes are required to get the attachment with the sphere in working order.

Preliminary experiments to determine optical properties of lettuce (Lactuca sativa L.) leaves, in particular absorption, using this device and one described earlier [11] gave results in good aggrement with each other (Fig. 3, B).

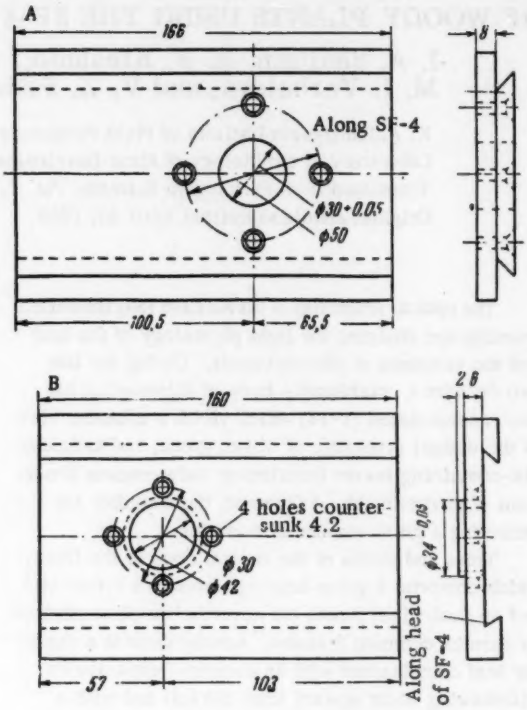

Fig. 2. Slit plate to SF-4. A) Slit plate to SF-4; B) to photocell housing mounted on the sphere.

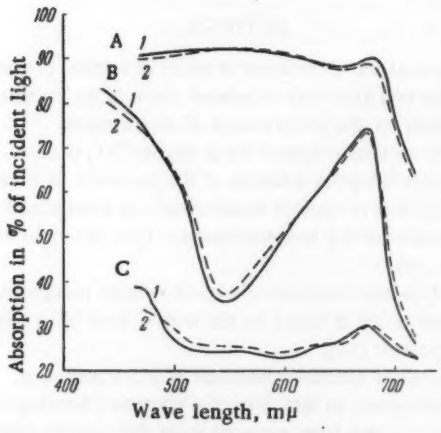

Fig. 3. Absorption of radiant energy by red leaves of Begonia rex (A), green leaves of Lactuca sativa (B), and white leaves of Acer negundo (C), obtained with an SF-4 spectrophotometer (1) and a KSA-1 spectrograph (2).

TABLE 1. Optical Properties of Bean Leaves as Affected by Short-Term Exposures to Various Temperatures (in % of incident radiation)

Wave length, mμ	Leaf temperature, °C	4 hr			1 hr			10 min*		
		transmittance	reflectance	absorption	transmittance	reflectance	absorption	transmittance	reflectance	absorption
545	1	18.5	15.5	66.0	18.0	16.0	66.0	19.5	17	63.5
	20	18.0	16.2	65.8	18.0	15.1	66.9	18.0	16.3	66.7
	40	18.5	17.5	64.0	17.0	16.2	66.8	17.9	16.0	66.1
760	1	45.0	45.0	10	47.0	45.1	7.9	43.1	48.8	8.1
	20	48.0	42.0	10	44.5	45.0	10.5	46.5	44.8	8.7
	40	40.5	46.5	13.0	46.2	46.8	7.0	47.9	47.0	5.1

*Leaves in this group were placed directly on ice.

TABLE 2. Characteristics of Leaves of Trees and Shrubs

Plant species	Leaf thickness, μ	Wt. per unit area, g/cm²	Water content, %	Chlorophyll content, g/cm²			
				a	b	a+b	a/b
		10^{-3}		10^{-5}	10^{-5}	10^{-5}	
Willow with ordinary leaves	165	15.2	51.4	2.41	0.67	3.09	3.60
White poplar	183	16.9	68.2	3.27	1.02	4.29	3.21
Willow with shiny leaves	183	—	—	1.81	0.86	2.67	2.11
Birch, shaded leaf	146	12.8	—	2.76	0.95	2.71	2.91
The same, leaf in the light	146	16.7	—	2.53	0.56	3.09	4.53
Mt. ash, old glabrous leaves	183	16.5	61.5	3.37	0.97	4.34	3.49
Same, young pubescent leaves	202	14.3	63.4	1.60	0.54	2.14	2.96
Apple	183	17.7	61.0	2.57	0.80	3.37	3.22
Russian olive, silvery leaves	220	17.3	68.2	3.70	1.01	4.71	3.67
Linden	165	11.5	60.7	2.80	0.83	3.03	2.65
Plane tree, green leaf	92	9.1	55.6	2.56	0.91	3.47	2.82
Plane tree, red leaf	183	17.8	65.7	3.40	1.12	4.52	3.03
Berberis, green leaf	367	19.5	73.4	2.47	1.08	3.55	2.29
Same, red leaf	238	14.9	69.1	2.96	0.91	3.47	2.82
Hazelnut, green leaf	183	14.1	65.8	1.39	0.68	2.07	2.05
Same, red leaf	165	11.9	70.9	1.93	0.97	2.90	1.99
Mock orange, green leaf	128	13.4	81.2	0.98	0.32	1.30	3.05
Same, yellow leaf	146	15.5	82.4	0.48	0.18	0.66	2.67
Oak	220	8.0	—	2.84	0.99	3.83	2.87

TABLE 3. Absorption of Radiant Energy by Leaves of Trees and Shrubs (in % of incident light)

Plant species	Wave length, mμ						
	460	480	540	600	670	680	740
Linden	92.8	91.7	78.7	84.0	91.9	88.5	24.7
Oak	84.2	83.0	66.7	71.0	79.5	78.4	6.0
Maple	85.8	85.8	75.5	75.7	74.8	75.7	6.0
Hazelnut	87.7	86.5	65.8	75.2	84.7	84.0	4.7
Plane tree	90.7	91.0	73.5	82.5	91.7	90.4	4.9
Mountain ash	92.5	92.5	79.4	84.2	91.2	92.4	10.2
White poplar	93.0	92.5	83.2	89.0	92.8	93.9	13.3
Apple	91.8	91.8	75.2	83.0	91.1	91.9	9.0
Birch	91.0	91.7	75.3	85.0	90.0	90.8	12.0
Mock orange	89.0	88.2	54.3	68.0	88.0	84.2	10.0
Berberis	93.0	93.0	73.0	80.8	93.5	92.7	12.0
Willow	93.6	93.8	82.8	89.3	94.5	94.0	13.0
Willow, shiny leaf	91.0	91.4	83.8	88.6	92.7	93.3	12.0
Russian olive	85.6	86.0	74.7	82.0	86.0	86.3	8.0
Average	90.1	89.9	74.4	81.3	88.7	88.3	11.6
±	2.6	2.7	5.4	5.1	3.7	4.7	4.0

The slight difference between curves is due to individual variations in the leaves. Similar data were obtained for the white leaves of box elder (Fig. 3, C) and the red leaves of begonia (Fig. 3, A).

Inasmuch as we were studying leaves of plants in natural conditions, where they were subjected to the effect of rapidly changing environmental conditions (for example high temperatures, cloudiness, bright sunlight, etc.), the question arose whether they, i.e., their optical system, would immediately reflect such changing conditions by an alteration of the optical system itself.

Leaves of bean plants (Vicia faba L.) grown in the greenhouse at 18-20° were divided into three groups: Plants of group I, with roots in the soil and without soil, were kept in a refrigerator at +1° for 4 hours, 2 hours, 30 minutes, and 10.5 minutes; plants of group II (controls) were kept at 18-22°; plants of group III were kept at +38-42° for 2 hours, 0.5 hour, etc.

Since leaves have a poor heat conductivity, it may be assumed that during the 10-20 seconds required to make measurements in one spectral region their temperature will change but little. Slides on which the leaves were placed for measurement were at the same temperature as the leaves, i. e., -1 to +1, 18 to 20, and 38 to 42°.

We chose two regions of the spectrum, 550 and 760 mμ. Results summarized in Table 1 show that in the cold group there are no differences in transmittance, reflectance, or absorption with length of exposure to low temperature. In both spectral regions studied, the green and the near infra-red, no substantial differences in absorption with temperature were noted. This is incontrovertible evidence that changes in colloidal structure of the protoplasm, protein complexes of the plastid, etc., cannot over short periods have any effect on the leaf's optical properties, as determined primarily by pigment content and anatomical structure [13].

The agreement of results obtained by different methods in preliminary experiments permitted the study of optical properties of woody plants by the present method.

The following plants were studied: linden (Tilia vulgaris L.), oak (Quercus robur L.), maple (Acer tegmentosum Max.), hazelnut (Corylus avellana L.), plane tree (Acer pseudoplatanus L.), mountain ash (Sorbus aria Grantz), white poplar (Populus alba L.), apple (Malus communis L.) birch (Betula verrucosa Ehrh.), mock orange (Philadelphus coronarius L.), willow (Salix sp.), and Russian olive (Eleagnus argentea Pursch).

Water content, chlorophyll content, and other items having a bearing on radiant energy absorption were determined at the same time that optical properties were being studied. Content of chlorophylls a and b was determined with the SF-4 spectrophotometer.

RESULTS

Various leaf characteristics are evaluated in Table 2. As the table shows, leaf thickness varies from 92-367 μ, with an average thickness of 170 μ. Weight per unit area varies from 8.0 to 19.5 mg/cm^2. Water content (in % of wet weight) varies from 51-82%, with an average of 66%. With respect to thickness and weight per unit area, the leaves are typically mesophytic in character, while with respect to water content they are xerophytic. Total chlorophyll content averages 2.01 mg/g, or $3.3 \cdot 10^{-5}$ g/cm^2.

Optical properties of the leaves are shown in Figs. 4-7 and Tables 3-4. Experimental data for individual species are presented in the tables.

Transmittance of radiant energy in the plants studied (Fig. 4, T) is minimal in the blue (400-500 mμ) and red (660-680 mμ) regions of the spectrum, being 1-3%, and maximal in the green (12%) and near infra-red (43%) regions of the spectrum.

The reflectance curve is similar to the transmittance curve, but is characterized by higher values in all regions of the spectrum (Fig. 4, R). The predominance of reflectance over transmittance testifies to the xeromorphic character of the leaves of woody plants.

Absorption of radiant energy (Fig. 4, A) is maximal in the blue (460-500 mμ) and red (660-680 mμ) regions, being 90 and 88%, respectively. It is minimal in the green region, being 73%. Scattering in the region of the maxima is 3-4% and in the region of the minimum, 6%.

Absorption of radiant energy, which depends mainly on chlorophyll content, may be expressed also by an optical density curve (Fig. 4, E), which is an index of the surface concentration of leaf pigments. Optical density in the blue and red regions is 0.85-1.15 and 0.80-1.10, respectively, and in the green region, 0.50-0.67.

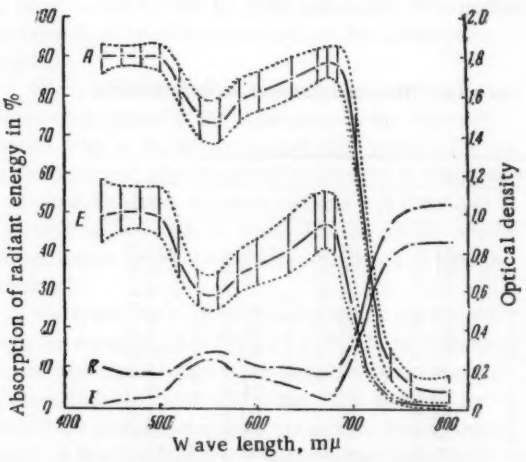

Fig. 4. Optical properties of leaves of trees and shrubs (average for 14 spp.). A) Absorption of radiant energy; R) reflectance; T) transmittance; E) optical density.

Among the trees and shrubs there are quite often encountered closely related species whose leaves are morphologically different. In Fig. 5 are shown the optical properties of dull and shiny willow leaves, both with a xerophytic structure. In spite of differences in surface structure of the leaf blade, reflectance and transmittance, and in consequence absorption as well, are practically identical in the normal and the shiny leaf.

Where there is a dense white appressed pubescence, this is manifested in the leaf's optical properties. In Fig. 6 are presented curves for leaves the lower side of which bears such a pubescence. Transmittance of radiant energy is small in an absolute sense and is the same for light incident on either side, being 2-3% at the minimum and 7-8% at the maximum (540-560 mμ). Large differences in reflectance are observed; the upper surface of the leaf reflects at 480 mμ and 670 mμ, 6 and 5% respectively, while the lower side reflects 33.5 and 32.5%, respectively. In the green region of the spectrum, reflectance by a pubescent leaf (Fig. 3, R2) is almost 40%.

Because of the differences in reflectance by the upper and lower sides of white poplar leaves, there are large differences in absorption, although the curves are qualitatively the same. In all regions of the spectrum the pubescent side of the leaf absorbs 25-28% less radiant energy than the normal green side (Fig. 6, A2).

A similar pattern is observed in plants in which the young leaves are pubescent and the mature leaves are either typical green leaves or are glabrous and shiny, as in mountain ash (Fig. 7). Here the young, more mesophytic leaf transmits more radiant energy (14%) in the 540-560 mμ region than the mature leaf (9%); these properties are due solely to anatomical and age factors, not to the character of the leaf surface, as indicated by the transmittance values for white poplar (Fig. 6).

Reflectance is also greater in the young leaf, but this is due to the pubescence, since leaves of the same

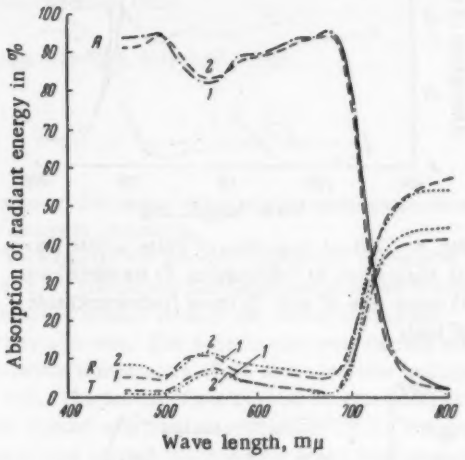

Fig. 5. Optical properties of willow leaves. A) Absorption; R) reflectance; T) transmittance; 1) dull leaf; 2) shiny leaf.

TABLE 4. Absorption of Solar Radiation by Leaves of Trees and Shrubs (in % of incident light)

Plant species	Physiological radiation, 400-720 mμ	Near infrared radiation 720-800 mμ
Willow with ordinary leaves	88.6	12.5
White poplar, absorption from the upper side of the leaf	88.6	12.5
The same from the lower side	59.4	13.1
Willow with shiny leaves	85.0	12.5
Birch, shaded leaf	85.0	16.7
The same, leaf in the light	78.8	8.4
Mountain ash, old glabrous leaf	82.5	8.4
The same, young pubescent leaf	70.0	12.5
Apple	81.3	12.5
Russian olive, silvery leaved	80.5	12.5
Linden	80.5	25.0
Plane tree	79.7	16.7
Berberis	78.8	8.4
Maple	76.1	8.4
Mock orange, green leaf	71.0	8.4
Mock orange, yellow leaf	38.7	8.7
Hazelnut	74.4	8.4

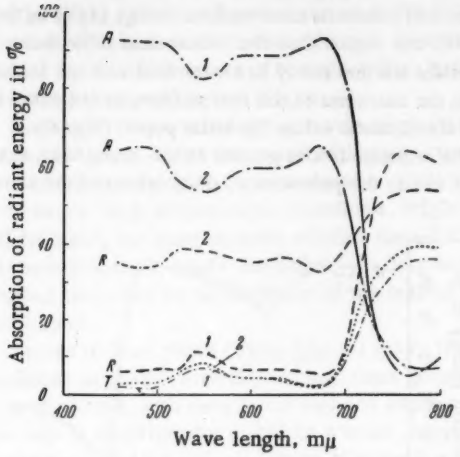

Fig. 6. Optical properties of white poplar leaves. A) Absorption; R) reflectance; T) transmittance; 1) upper side of leaf; 2) lower (pubescent) side of leaf.

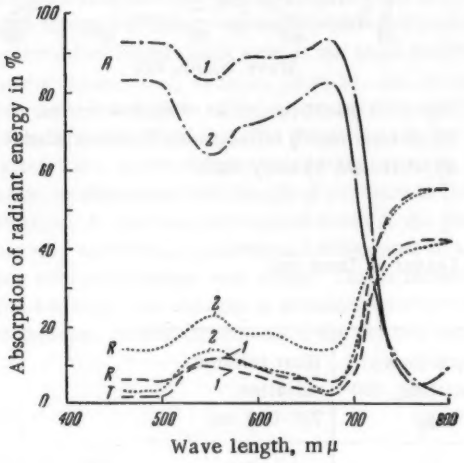

Fig. 7. Optical properties of mountain ash leaves. A) absorption, R) reflectance; T) transmittance; 1) old (glabrous) leaf; 2) young (pubescent) leaf.

age reflect strongly from the pubescent surface (Fig. 6). Absorption by young and mature leaves varies accordingly (Fig. 7).

Data on absorption of physiological radiation (400-720 mμ) in direct sunlight in midday are presented in Table 4. The table shows that, with the exception of young leaves (mountain ash) and extreme mesophytes (mock orange) or plants which grow in the shade as a rule (hazelnut), absorption in the majority of species averages 80%.

SUMMARY

The optical systems of leaves of the majority of woody plants from the central region of the USSR are practically identical; this is due to a homogeneity in leaf structure and to similar chlorophyll content levels. Leaves of woody plants are distinguished by a somewhat xerophytic character.

The similarity of leaves of woody plants with respect to their optical properties and their xeromorphic character is undoubtedly due to the nature of the water uptake mechanism (raising of water to great heights) and to a greater insulation than in herbaceous plants.

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THE EFFECT OF PHYSIOLOGICALLY ACTIVE SUBSTANCES ON THE REPRODUCTION OF TOBACCO MOSAIC VIRUS

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At the present time it has been established that the reproduction of viruses is closely connected with the character of the metabolism of the organism [1, 2].

The widespread use of physiologically active substances for such purposes as fighting weeds, retarding the sprouting of tubers, root crops, and bulbs in storage, for regulating the processes of fruit formation, decreasing the fall of fruit prior to ripening, using these substances as defoliators, desiccants, etc., is based on the change in metabolism in one or another direction. The change of metabolism originating under the effect of chemical agents must have an effect on the character of reproduction of plant viruses.

There are only individual indications of the effect of physiologically active substances on the accumulation of viruses in the plant in the literature. It is noted, for example, that 2,4-dichlorophenoxyacetic acid (2, 4-D) inhibits the buildup of potato virus X and Y [3]. There are indications of the connection of the reproduction of plant viruses with the concentration of auxins in the plant tissues. Pavillard [4] established that a decrease in the concentration of auxins is observed with a buildup of viruses. In connection with this, it is interesting to note the character of the distribution of viruses in the leaves of the different levels. In such parts of the plant as the young growing leaves and the point of growth, which are, as is well known, richest in auxins, viruses are found in a significantly smaller quantity, and sometimes they are not observed at all [5]. The most intensive buildup of viruses is noted in the leaves of the middle stage. In the leaves of the lower level, in which auxins are less concentrated than in the other leaves, and their activity is sharply decreased in strength, the accumulation of viruses proceeds less intensively.

At the present time there are few data on the basis of which one could judge the effect of various physiologically active substances on the reproduction of viruses in the plant. At the same time, the rate of use of chemical growth stimulators in agriculture is increasing more and more. In connection with this, an explanation of the effect of physiologically active substances on the reproduction of phytopathogenic viruses is extremely necessary.

We carried out experiments on the effect of 2, 4-D, 2, 4, 5-T, gibberellin, and heteroauxin on the buildup of tobacco mosaic virus in the leaves of tobacco, variety Mamont. The experiments were carried out at a constant temperature of 25° and a relative humidity of 70%. The leaves of control and experimental plants were lighted with luminescent lamps (DC). The experiments were carried out on leaves taken from completely healthy plants. Examination of the leaves for infection with tobacco mosaic virus was done under an electron microscope. Young leaves (immature), leaves of the middle level (mature), and leaves of the lower level (old), were included in the experiment. Inoculation was done with pure preparations of tobacco mosaic virus, diluted to a specific concentration and with the help of the rough surface of a glass pestle, the inoculation of the virus preparation was rubbed on the upper side of the leaf. Fifteen minutes after the application, the leaves were washed with ordinary tap water for a period of five minutes. Washing was carried out with the goal of removing from the surface of the leaf the virus particles that did not pass into the tissues. After the leaves were dried with filter paper, small pieces were cut from them so that there were parts of the left and right sides of the leaf in each of the variants. Young leaves were cut in two along the central vein and the entire halves of the leaf were used in the experiment. The halves and pieces of leaves of the experiment variants were placed in solutions of physiologically active substances, used in specific concentrations, and each corresponding control variant was placed in distilled water. The concentrations of the solution of each substance tested was constant. This was accomplished by means of adding water to the container to the degree of its evaporation in the process of the experiment. All leaves were placed with the upper side on the surface of the solutions or water. This made it possible to ensure the assimilation of oxygen into the tissues through the stoma, located in the main part on the lower surface of the leaf.

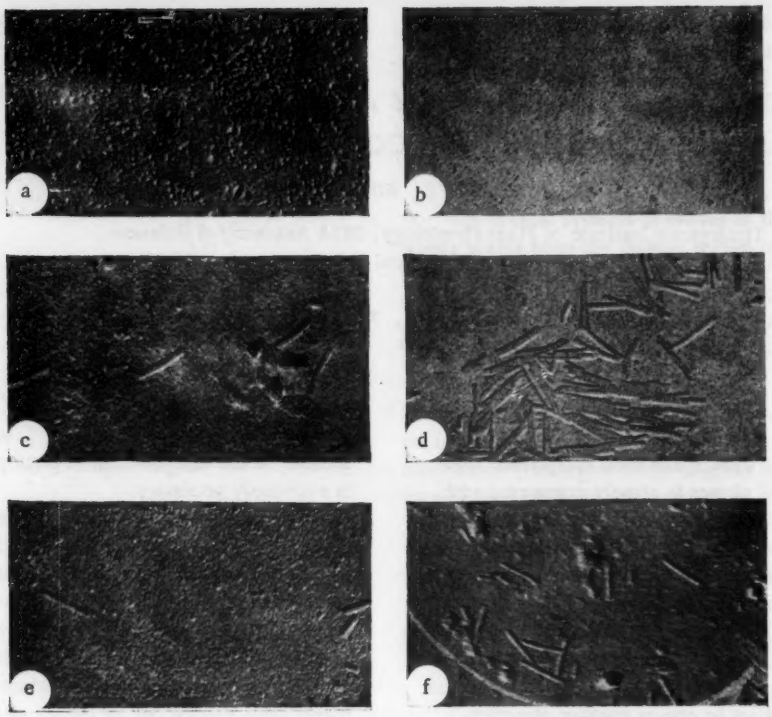

Fig. 1. 2,4-Dichlorophenoxyacetic acid (2,4-D). Young leaf: a) Control, b) 2,4-D; mature leaf: c) control, d) 2,4-D; old leaf: e) control, f) 2,4-D.

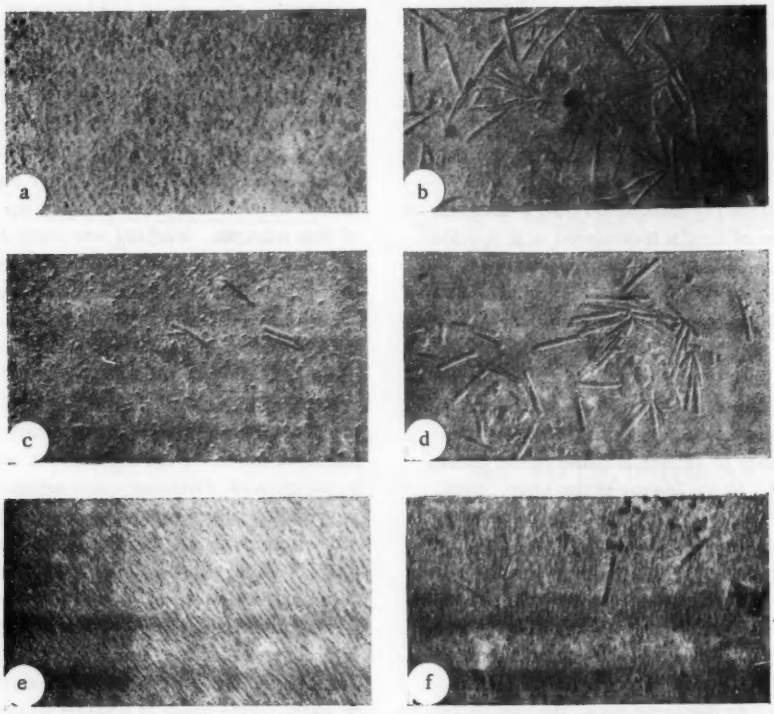

Fig. 2. 2,4,5-Trichlorophenoxyacetic acid (T). Young leaf: a) Control, b) T; mature leaf; c) control, d) T; old leaf: e) control, f)T.

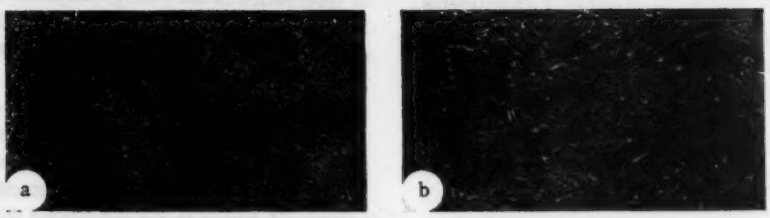

Fig. 3. Gibberellin. Young leaf: a) Control, b) gibberellin.

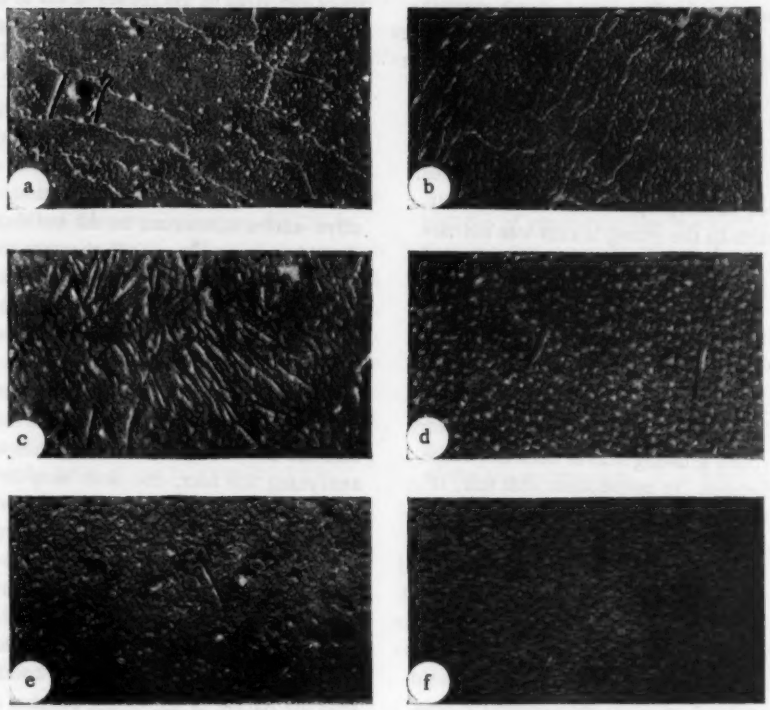

Fig. 4. Heteroauxin (IUK). Young leaf: a) Control, b) IUK; mature leaf: c) control, d) IUK; old leaf: e) control, f) IUK.

Carrying out of the experiments on inoculated sections and halves of leaves made it possible to exclude the effect of the physiologically active substances of the plants on the buildup of the virus, which made it possible to explain more clearly the effect of the test preparations.

Testing the effect of the growth stimulators on the reproduction of the viruses by means of establishment of the experiments on the entire plant did not make possible an appraisal to the necessary extent of the test preparations, nor the establishing of the character of the buildup of the virus in the leaves of the different levels, because of the movement of physiologically active substances of the plant from some organs to others, which is always taking place.

The quantity of virus accumulated in the leaves of each variant of the experiment and in the corresponding control was determined under an electron microscope. For this we took standard samples of ten sections from the left and right halves of the leaves of the experimental and control variants. After pulverizing the sections in a glass homogenizer until a pure mass was obtained and dilution of the ground material with distilled water up to the level 1:200, preparations were made for observations under the electron microscope.

The experiments on the effect of the highly reactive physiologically active substances were repeated

many times. The results of each series of experiments agreed completely. As an illustration, we present the data for the experiment carried out in the period from December 30, 1958 to January 15, 1959.

2,4-Dichlorophenoxyacetic Acid

This preparation is used widely in agriculture at the present time as a herbicide. The nature of the action of this substance on the plant is such that when a solution of this preparation falls on the point of growth and on immature leaves, a clear picture of changes is observed which by its external appearance reminds one of the changes observed on inoculation of plants with tobacco mosaic virus. The action of this preparation was tested at a solution concentration of 25 mg/liter (Fig. 1). As the picture shows, 2,4-dichlorophenoxyacetic acid significantly stimulates the reproduction of the tobacco mosaic virus in the leaves of the middle and lower layers. This preparation does not change the process of the buildup of the virus in the young leaves. Both in the presence of the preparation and without it, a buildup of the virus in the young leaves was not observed. The most intensive buildup of the virus in the presence of the preparation took place in the leaves of the middle layer.

2,4,5-Trichlorophenoxyacetic Acid

In the absence of 2,4-dichlorophenoxyacetic acid, this preparation when applied in a solution to the point of growth and to young growing leaves does not cause sharp formative changes. In connection with this, it would be interesting to compare the effect of this preparation on the reproduction of the tobacco mosaic virus with the action of 2,4-dichlorophenoxyacetic acid on this process. The 2,4,5-trichlorophenoxyacetic acid was tested at a concentration of 28.6 mg/liter, which in a molar relationship corresponded to the test concentration of the solution of 2,4-dichlorophenoxyacetic acid. Figure 2 shows that 2,4,5-trichlorophenoxyacetic acid increases the buildup of the virus in the leaves of all levels. The greatest stimulation of the buildup is observed in the young leaves and leaves of the middle layer.

Gibberellin

This substance is a new, highly active stimulator of plant growth. The strongest answering reaction of the plant to a treatment with gibberellin is observed in the case where the solution of this substance is placed on the point of growth. The action of gibberellin results in formative changes of the leaves and stem, expressed by a lengthening of the leaf blade, sharp elongation of the stem and the appearance of mosaic coloring of the leaf. The change in the reproduction of the tobacco mosaic virus was studied with a solution concentration of 200 mg/liter. In Figure 3, data showing the sharp stimulation of the buildup of the virus under the effect of gibberellin in the young leaves are presented. Noticeable changes in the buildup of the virus were not noted in the leaves of the middle and lower levels.

Heteroauxin

As we have indicated above, the reproduction of viruses is sharply inhibited in the parts of the plant richest in auxins. It would be interesting to study the buildup of viruses in the inoculated leaves with an artificial enriching of the latter with heteroauxin. The action of the heteroauxin was tested using a solution concentration of 100 mg/liter. Figure 4 shows that heteroauxin to a significant degree decreases the buildup of the virus in the leaves of all levels. Heteroauxin shows the sharpest effect on the leaves of the middle and lower levels, which contain significantly less of the natural auxins than the young leaves.

The investigations that were carried out showed that the various physiologically active substances show dissimilar action on the reproduction of the tobacco mosaic virus. Comparing the action of the tested formative-active substances on the buildup of the virus and those formative changes in the growing organs of the plants, which appeared under the effect of treating the plants with these preparations (Fig. 5), one cannot note a specific interrelationship. The 2,4-dichlorophenoxyacetic acid, as was already suggested above, causes strong formative changes in the plants. This same substance shows a depressing action in the buildup of the tobacco mosaic virus in the immature leaves. When analyzing this fact, one must keep in mind that the formative changes taking place under the effect of this substance bring to mind the picture of the changes taking place when the plants are inoculated with tobacco mosaic virus. This is important because the phenomenon of interference of viruses was established long ago in virology, in which the presence of one virus in an organism inhibits the development of another [2]. We propose that the nature of the effect consists of the fact that changes in metabolism taking part as a result of the buildup of one virus are inhibiting for the buildup of another virus.

Assuming that with the interaction of the two viruses, a shift in metabolism taking place under the effect of the first is inhibiting for the buildup of the second virus, one can also assume that in our case, the effect of the interference between the action of the formative-active substance on the organism and on the buildup of the virus is noted. These changes in metabolism, which must take place as a result of the action of the virus on the cell, also took place under the effect of the physiologically active substance. In the given case, the physiologically active substance emerges in the role of a unique antimetabolite with respect to the virus particles. This effect can take place only in the case where the virus particle enters into tissue whose metabolism has already been changed in the same direction in

which it would be changed as a result of the movement of the virus particle into the cell. In the case of the action of 2,4-dichlorophenoxyacetic acid on the mature leaves and on the leaves of the lower level (old) a stimulation of the virus reproduction is noted. The stimulation of the buildup of the virus in this case very likely is explained by the total of the similar action on metabolism of the virus and of this substance. This is possible in the case where changes in metabolism taking place under the effect of this substance would take place simultaneously with changes taking place under the effect of the virus. The metabolism of the leaves of middle and lower levels is differentiated by great stability in comparison to the metabolism in the young, growing leaves. Shifts in the metabolism of the young leaf take place earlier than in leaves of the middle and lower levels. This evidently also explains the inhibiting of the buildup of the virus in the young leaves and the stimulation of reproduction in the middle and lower levels.

Another substance from the group of substances that were studied that causes formative changes similar to the changes taking place under the effect of the tobacco mosaic virus is gibberellin. This substance is less active with respect to its formative action. In this connection, it is of interest to consider the stimulation of reproduction of the tobacco mosaic virus in the young leaf under the effect of gibberellin, in the absence of inhibition of this process under the effect of 2,4-dichlorophenoxyacetic acid. The stimulation of the virus reproduction in this case evidently takes place as a result of the total action of the substance and of the virus, as already noted in the case with 2,4-dichlorophenoxyacetic acid in the old and mature leaves. Such an action of gibberellin on the young leaves is probably explained by the slower shifts in metabolism, in comparison to 2,4-dichlorophenoxyacetic acid, to the side of those changes which take place under the action of the virus. Under these conditions, the action of the virus and of the substance on metabolism takes place simultaneously.

We carried out a special experiment with the goal of checking the effect of the interference of the virus and of the formative-active substance on metabolism. The experiment consisted of inoculating formatively changing leaves of tobacco plants which had been under the effect of gibberellin. The tobacco mosaic virus did not build up in these leaves. Thus, the experiment confirmed the presence of interference between the action of the formative-active substance and of the virus on the metabolism. This might well be used as a basic treatment in the chemical battle with phytopathogenic viruses.

The increase in the buildup of the virus in the presence of 2,4,5-trichlorophenoxyacetic acid is apparently connected with the same effect of this preparation on metabolism as in the experiments with gibberellin and with the effect of 2,4-dichlorophenoxyacetic acid on the leaves of the middle and lower levels.

Experiments on the effect of heteroauxin on the buildup of viruses support the earlier noted relationship between the concentration in the tissues of this substance and the reproduction of the viruses. In order to establish the possible mechanism of the action of heteroauxin, it is necessary to remember that the reproduction of the viruses is determined by the condition of the metabolism of the plant itself. Of decided importance for the implantation into the metabolism of the virus nucleic acid, is the condition of the nucleic metabolism of the macroorganism. It is possible that the reproduction of the virus arises in that case where the metabolic activity of the nucleic acid in the plant falls in comparison to the activity of the nucleic acid of the virus. In this case the condition is created where the virus nucleic acid can visibly change the metabolism and effect a changeover in the synthesis of natural nucleoproteids corresponding to the specific characteristics of the

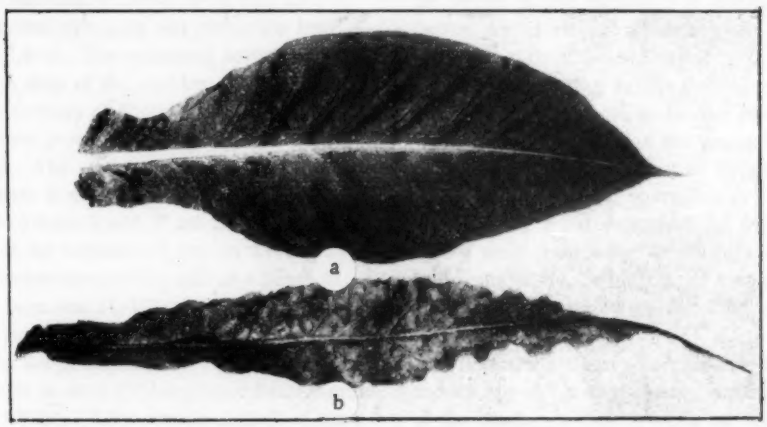

Fig. 5. Formative changes in tobacco leaves. a) Control; b) gibberellin.

nucleic acid of the virus [6]. Retarding or complete halting of the reproduction of the virus in the plant tissues enriched with auxins must be viewed as an indicator of the high metabolic activity of the nucleic acid of the plant itself [7]. Thus, one of the most important aspects of the part of heteroauxin in metabolism is its role as a stabilizer of the normal nucleic metabolism.

SUMMARY

1. Physiologically active substances used as growth stimulators and herbicides show an effect on the reproduction of phytopathogenic viruses.

2. Chemical substances differentiated by their formative action on the plant, which reminds one of the picture of formative changes usually taking place with virus diseases, in some cases retard and in others stimulate the buildup of the virus.

3. The effect of the interference between the effect on metabolism of formative-active substances and the effect of the virus was established. This effect consists of changes in the metabolism of the plant that are inhibiting for the buildup of the virus, which take place as a result of the action of the formative-active substance. This effect can be used as a basic treatment in the battle with phytopathogenic viruses.

4. In the plant tissues rich in auxin, the buildup of viruses is decreased or does not take place at all. Additional enrichment of the plant tissues with auxins halts or decreases sharply the buildup of viruses. Heteroauxin, while increasing the metabolic activity of the nucleic acids in the plant and creating by this an obstacle to the inclusion of the virus nucleic acid in metabolism, plays the role of a stabilizer of the normal plant metabolism.

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THE DECOMPOSITION OF 2,4-D IN THE TISSUES OF CANADA THISTLE

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The battle with perennial weeds is usually most effective only in the case where their roots are destroyed. Strong, well-developed roots give the plant a great ability to live. The fact is that the root system not only supplies the plant with water and with mineral substances, but also forms complex organic substances necessary for normal activity [1-3].

Thus for example, sugars entering into the roots from the leaves after glycolysis and reactions of the Kreb's cycle are converted into organic acids. Some of these acids (pyruvic, oxalacetic, ketoglutaric) reacting with the ammonia nitrogen assimilated from the soil, are converted to amino acids. A biosynthesis of nucleic acids takes place in the roots [9]. Along with this, organic acids transmit soil carbon dioxide to the aerial organs, strengthening the carbon nutrition of the plants[1].

In order to explain several failures of field experiments with the herbicide 2,4-D, and also to improve the chemical method of fighting perennial weeds, it is necessary to trace the change of 2,4-D in their tissues.

We attempted to explain the character of the movement and distribution of the 2,4-D herbicide in the plants of Canada thistle (Cirsium arvense) in various phases of its development.

METHOD

Radioactive 2,4-dichlorophenoxyacetic acid held in a C^{14} carbon cycle (specific activity 2.02 mC/g) means of neutralization with soda was converted into the sodium salt of 2,4-D. The measured activity of the 2,4-D showed that a drop of the working solution (volume of 0.03 ml) had an activity of 6500 impulses/minute.

A small, separate group of Canada thistle was used in each experiment. The work was carried out on eight to ten plants (rosettes) in each experiment, and the results that were obtained (Tables 1 and 2) are the average of these. One series of the experiment was on thoroughly warmed soil (the temperature of the soil at a depth of 10 cm in May and June was 15-17°) and the other series was on a cool soil (soil temperature of 10-12°). Fifteen drops of the marked 2,4-D solution were placed on the leaves of each rosette or stem (Vyborg State Farm, Leningrad District, 1957-1958). After certain periods of time (see tables), vertical (to a depth of 100 cm) and horizontal (to 50 cm) excavations of the roots were carried out and also the above-ground portion of the Canada thistle was cut. The above-ground mass and the roots were carefully washed.

Then part of the sample was dried and analyzed on an end counter for β-rays. The background radioactivity and the experimental radioactivity were measured for a period of five minutes. There were three repetitions of the measurements. From the other part of the sample, the cell sap was squeezed and young plants of garden cress (Lepidium sativum) were sprayed with this sap.

The reaction of the garden cress (death, inhibited growth, normal vegetation), together with the radioactive count, made it possible to judge the presence or absence of 2,4-D (or products of metabolism similar in their toxicity) in the plant sample (Table 1).

Inhibited growth of the garden cress suggests the presence of 2,4-D residuals in the plant extract and is designated by a plus sign; normal vegetation suggests the absence of 2,4-D and is designated by a minus sign (Table 2). We will look at the data of the first experiment (Table 1).

We found in the experiments that the absorption and movement of 2,4-D changes with the phases of development of the plant and depends on the soil temperature. The main part of the marked 2,4-D remained in the above-ground portion of the Canada thistle on the plots with a decreased soil temperature. A similar picture is observed on well-warmed soils only in the early spring period (rosette phase). When the plants are treated in the period of bud formation or during the autumn rosette period, the main part of the marked 2,4-D appears in the roots of the Canada thistle.

It is interesting to note that marked 2,4-D practically does not enter into the horizontal roots on the cool soils, while noticeably more of the marked 2,4-D enters into the lateral roots of the Canada thistle on the well-warmed soils. However, these quantities of 2,4-D cannot be equal to those that move into the vertical roots. Similar results were obtained by Crafts [4] using the method of radioautography. With this, of course, it is difficult to express quantitatively the distribution of the 2,4-D herbicide in the plant.

It is not difficult to notice that the absorption and movement of 2,4-D is closely connected with the activity of the plants. In spring, when the reserve substances of the roots move into building the aerial mass of the plants, the 2,4-D herbicide in the main part remains in this aerial mass. During the period of bud formation, part

of the assimilates is needed in building the generative organs, but part moves into the root system and here undergoes complex transformations [1-3]. With the increased movement of assimilates, the assimilation of marked 2,4-D into the roots of the plants is also increased. This underlines the relationship of the movement of 2,4-D to the movement of the products of photosynthesis [5].

The autumn treatment of the plants acted in a similar manner, when the assimilates move increasingly into the roots of the plants [6] and are deposited there in the form of reserve substances; simultaneously, an increased movement of the marked 2,4-D into their roots takes place.

In connection with the data that were obtained, it must be noted that, as earlier investigation showed [7], synthetic compounds used as stimulators and herbicides flow with greatest intensity to those parts of the plant that are characterized by the most intensive metabolism.

It was also found in the experiments that the rate of decomposition of the 2,4-D herbicide in the roots of Canada thistle depends on its activity (Table 2). The fact that in several experiments low radioactivity was observed at the same time that there was strong suppression of the garden cress can be interpreted as meaning that the decomposition of 2,4-D does not take place here, but that it is connected with the products of the plants' activity [8]. This does not exclude the possibility that as a result of the decarboxylation of the marked 2,4-D, a rapid loss of the $C^{14}O_2$ takes place as a result of the abnormal metabolism of the thistle plants.

With spring treatment of the rosettes of Canada thistle with marked 2,4-D, samples of its roots taken from a depth of 70-80 cm retained radioactivity up to 75 days. During this same period the cell sap squeezed from the roots strongly suppressed the growth of the garden cress. In the spring of the following year, samples of the roots had already lost radioactive substances, and, correspondingly, the cell sap from the roots did not possess phytotoxicity.

A similar picture was observed also for autumn-treated Canada thistle. However, in the latter case, samples from the roots contained traces of radioactive substances (11.7 impulses/minute per 50 mg of dry weight of the roots) in the spring of the following year. Exactly the same cell sap from the roots of the thistle noticeably inhibited (62.4%) the growth of the garden cress in the following spring. Consequently, when spring and autumn rosettes of Canada thistle are treated with marked 2,4-D, the latter is retained in the roots of the plant in small quantities for a period of several months. Concerning the treatment of Canada thistle during the period of bud formation, radioactive substances already have disappeared from the roots after 30 days from the beginning of the experiment and the cell sap from the roots does not suppress garden cress. In this case, the decomposition of combining of the 2,4-D takes place relatively rapidly. This agrees completely with the data of other investigators [9].

Such a decomposition or combining of the 2,4-D at the beginning seems strange. However, if it is remembered that in the experiments with the spring and autumn rosettes, the 2,4-D herbicide remaining in the above-ground mass or moving into the roots is insufficiently energetically drawn into the metabolism, then the long length of retention of 2,4-D in the plant tissues in understandable. On the other hand, from experiments performed in the period of bud formation, when the various processes of metabolism are proceeding energetically, 2,4-D is drawn into the cycle of transformation rapidly and soon is decomposed or combined.

In this connection it can be shown on an earlier established rule that with an increased intensity of metabolism the chemical conversion and detoxification of the toxic substances which moved into the plants are significantly accelerated [10, 11].

It is necessary to stress that far from a direct relationship between the duration of the retention of 2,4-D in the roots of the plant and the degree of mortality of the Canada thistle in the field experiments is always observed (Table 3). This relationship seems clear when the autumn rosettes are treated. Here the great duration of retention of 2,4-D in the roots would seem to aid the weaker growing of the Canada thistle rosettes. However, with treatment of the spring rosettes, good growth of the rosettes of the plant is observed in spite of the long duration of the

TABLE 1. The Absorption and Movement of 2,4-D after Spraying Plants of Canada Thistle (impulses/minute per 50 mg of dry substance three days after the beginning of the experiment)

Phase of development at the time of treatment	Plots with decreased soil temperature			Plots with well-warmed soil		
	leaves	vertical roots	horizontal roots	leaves	vertical roots	horizontal roots
Spring rosette	135.3	0	0	117.0	83.0	0
Period of bud formation	252.0	14.7	0.4	176.0	196.6	27.3
Autumn	105.0	12.8	1.0	151.0	244.0	36.6

TABLE 2. The Rate of Movement of 2,4-D in the Roots After Spraying Canada Thistle at Different Stages of Its Development

Phase of development at the time of treatment	After 15 days		After 30 days		After 45 days	
	impulses/ minute per 50 mg of dry substance	reaction of the garden cress	impulses/ minute per 50 mg of dry substance	reaction of the garden cress	impulses/ minute per 50 mg of dry substances	reaction of the garden cress
Spring rosette	7.0	+	15.3	+	90.3	+
Period of bud formation	16.7	+	0	–	0	–
Autumn rosette	33.1	+	10.7	+	8.1	+

TABLE 3. Death of Plants of Canada Thistle and Death of Its Rosette after Spraying with 2,4-D in the Quantity 3 kg/hectare

Phase of development at time of experiment	Percent of dead plants 35 days after spraying		Percent of growing rosettes one year after spraying	
	cool soil	warm soil	cool soil	warm soil
Spring rosette	3.3	97.1	87.7	78.0
Bud formation	57.4	85.0	91.1	63.7
Autumn rosette	61.5	100	78.0	56.3

2.4-D retention in the tissues of the Canada thistle in the spring of the following year.

It is characteristic that with treatment of Canada thistle in the period of bud formation, a less intensive growth of the rosettes is observed than with treatment during the spring rosette phase. It follows from this that the high mortality of the thistle from the 2,4-D herbicide depends not only on the duration of retention of the latter in the plant tissues, but also on the condition of the root system. If the root system is dormant (when its active growth has finished) then it stays alive in spite of the presence of 2,4-D in it.

SUMMARY

1. It was established that the assimilation, movement in the roots, and decomposition of 2,4-D in the tissues of Canada thistle depends on the period of its vegetation, soil temperature, and condition of the root system.

More 2,4-D passes both into the vertical and into the horizontal roots on the warm soils than on cool soils. The decomposition of 2,4-D proceeds fastest of all during the period of bud formation of the thistle. If the root system is dormant, then the 2,4-D is not able to suppress or destroy the Canada thistle.

2. With spraying of spring rosettes, the main part of 2,4-D herbicide remains in the above-ground mass of the thistle. Insignificant quantities of 2,4-D move into the roots. With this, the decomposition of the 2,4-D takes place not earlier than two-and-a-half months. This guarantees a high mortality of the Canada thistle rosettes immediately after such treatments. However, in the spring of the following year, very many of the rosettes of the thistle are growing on such plots.

3. Spraying plants during the period of bud formation aids in the significant uptake of 2,4-D into the roots of Canada thistle on warm soils. On cool soils an insignificant quantity of 2,4-D moves into the roots of the thistle. During this period a rapid decomposition of the 2,4-D takes place by the tissues of the thistle (20-30 days).

Treatment of plants during this period results in a less intensive mortality of the aerial mass in the given year; however, in the following year fewer rosettes grow on such plots than on the spring-treated plots.

4. Spraying of autumn rosettes of Canada thistle aids the increased uptake of 2,4-D into the vertical and horizontal roots of the plant. With this, part of the 2,4-D is retained in the roots until the spring of the following year. This results in high mortality of the Canada thistle roots.

5. The competition of the action of the 2,4-D on the roots and on the aerial mass of perennials, and also the difference between the dying of the thistles and the rate of decomposition of 2,4-D in the plant roots, makes it possible to assume that the process of biological des-

truction of 2,4-D also is a process of the beginning action of the herbicide on the plant, when the 2,4-D fragments are competively absorbed by the plant cell receptors.

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GROWING PLANTS IN GRAVEL FOR RESEARCH PURPOSES

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In the study of plant nutrition, physiologists long ago gave up soil for a simple inert medium like sand in water with nutrient salts added. In this way data are obtained concerning the importance of various mineral elements to plant life. Recently those practicing gravel culture have recommended periodic irrigation of the inert substrate with nutrient solution [1]. This technique has been extensively employed by plant physiologists in experimental work. We have used it in two ways at the Artificial Climate Station; for growing plants in greenhouse vessels (Fig. 1) and in small plots 1570 × 1300 cm (Fig. 2).

The greater ease of aeration of the plant root system is an advantage of the gravel culture technique compared to water or sand culture. The salt solution can flow out, permitting air to fill all of the pore space. Mineral compounds needed by the plants are assimilated from the nutrient mixture which remains as a liquid film on

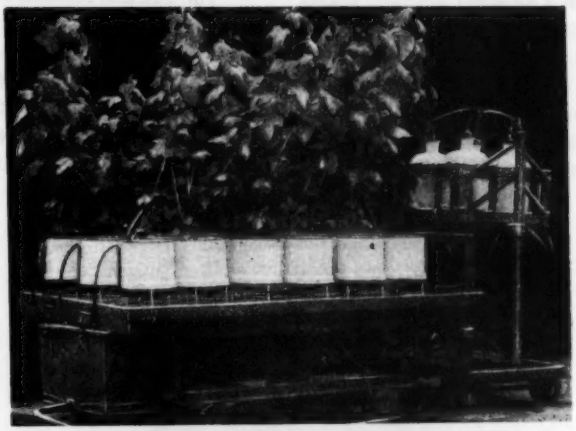

Fig. 1. Black Currant (Ribes nigrum) growing in gravel-filled vessels on a truck; right–nutrient solutions on a cart.

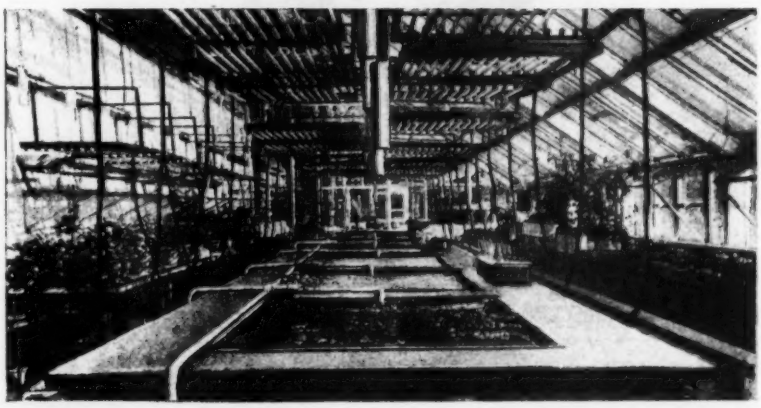

Fig. 2. Gravel-culture plots in the center of a greenhouse; above – fluorescent lights.

the root surfaces and around each gravel particle. Therefore, the absorption of nutrient elements in the presence of sufficient air is assured.

In gravel culture the root system can also be removed from the substrate with a minimum of damage (Fig. 3). During a study of the frost resistance of detached branches we found that, although it is better to work with entire plants, the latter are difficult to get into a refrigerated chamber in sufficient number when the root system is in soil. In order to avoid this clumsy situation we changed to the raising of plants in gravel and removing the root system from a plant prior to cold treatment. This allowed us to subject the shoot and root portion to various negative temperatures and to use a larger number of replicates. After the plants were thawed out they were planted in soil and further observations of their growth were made. We have tested this method for the determination of frost resistance of woody species and it seems to be reliable and convenient.

In gravel culture one salt solution can be readily exchanged for another. For example, it may be necessary to provide the plant with a different nutrition during various growth stages. In this technique the existing nutrient solution may be quickly removed, the substrate washed well with water and then the other nutrient solution applied to the plant roots. Gravel culture can also be recommended for the study of the plant resistance to salinity. In this way different types of salinity may be created (chloride, sulfate, and carbonate at various concentrations), coordinating them to certain growth stages of the experimental plant. At any time the plant may be returned to normal nutrition by washing the substrate with water. In gravel culture it is not difficult to arrange for the study of types of "soil" drought, i.e., to remove water from the rooting medium. In our experiments we used small grains of granite (3-7 mm). After the impurities were carefully washed out, the grains seemed quite inert. The circulating solution distributed itself more uniformly in the gravel than in sand.

In the greenhouse experiments enameled Mitscherlich vessels were used. An opening in the bottom admitted a rubber stopper fitted with a glass tee. To avoid fractures the latter should be made of metal or plastic. In the future we are going to devise special vessels for this. The replicate vessels (in our experiment, six) were joined to one another with rubber tubing (Fig. 4). The latter emerge from the bottom of the vessels; therefore the vessels must have a support of sufficient height.

The nutrient solution flows from the bottle into the vessels below. In order that the fluid is supplied to the vessels at a uniformly rapid, comparatively constant flow rate, a metallic tube of a certain diameter or a screw clamp is inserted in the line of each vessel. It is still necessary to regulate the depth of the solution used in the vessel. The solution should not reach the

Fig. 3. Left – Black currant plants grown in a gravel culture, right – root system of a similar plant removed from the substrate.

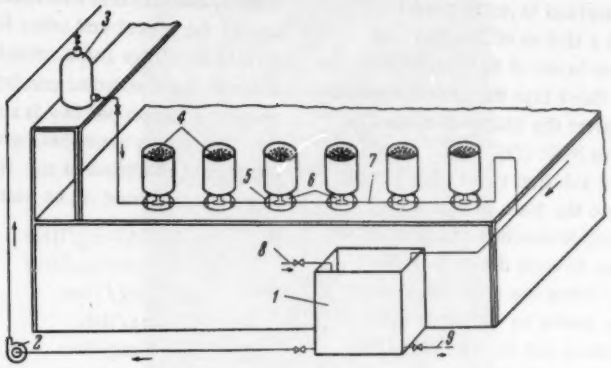

Fig. 4. Sketch of apparatus for raising plants in gravel-filled vessels in the greenhouse. 1) Tank for effluent solution; 2) pump; 3) bottle for nutrient solution; 4) vessels for plants; 5) vessel support; 6) T-joint; 7) rubber tubing; 8) tap from water line; 9) drain to sewer.

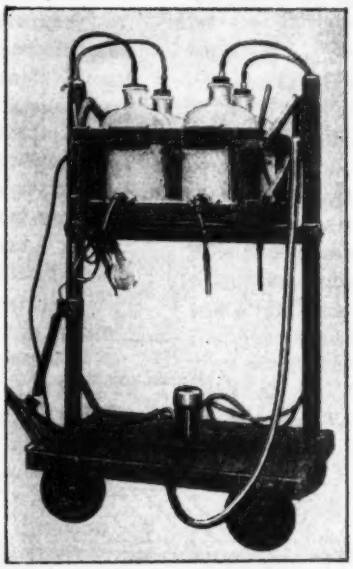

Fig. 5. Cart for conveyance and supply of plant nutrient solution.

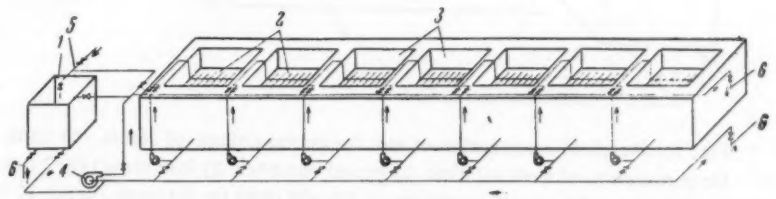

Fig. 6. Sketch of apparatus for raising plants in gravel culture in small greenhouse plots. 1) Tank for preparing fresh solution; 2) grid of perforated tubes to equalize fluid delivery; 3) gravel chamber; 4) pump; 5) tap from water line; 6) drain to sewer for spent solution.

surface of gravel in order that salt crystallization and algal growth do not occur in the surface layer of gravel. Regulation is achieved with a siphon of flexible hose which determines the highest level of the liquid (Fig. 1).

The supply of solution flows into the gravel through the rubber tube by gravity after the clamp is opened. In the greenhouse a shelf was built (Fig. 1 and 4) on which the bottles of nutrient solution stood with a tube from each bottle hooked into the train of tees and rubber tubes. When the solution reached the given level in the vessels, it drained out through the siphons into the tank beneath the shelf. From the tank the solution is again transferred into the bottle by a "Kama" pump. Thus the same nutrient solution can be used repeatedly for a week, after which it is changed for a freshly prepared solution. In our greenhouse experiments, about 400 liters of solution circulated through 4.5 tons of gravel.

In case the plants are raised on wheeled trucks, a special cart is brought up to the truck (Fig. 5).

The bottles of nutrient solutions are placed on the top shelf of the cart and after the tubes are connected up, the nutrient solution gravity feeds into the line serving one replicate series of vessels. When the solution reaches the determined level it begins to siphon into a tank suspended beneath the truck (Fig. 1). A pump on the lower shelf of the cart transfers the liquid from the tank back into the bottle for circulation through the gravel of another series of replicates. One cart is able in six hours to serve 20 trucks of plants giving nutrition to four experimental treatments simultaneously. How frequently the root system and gravel ought to be irrigated is determined by the size of the plants and vessels, the ambient external conditions, the coarseness of the gravel and other factors. For one-year-old apricot seedlings and a rooted cutting of black currant under Moscow summer conditions a single pass of nutrient solution per day is sufficient.

The plants mentioned grow well in Hellriegel solution as modified at the Station by A. F. Agafonova (figures calculated on an element basis):

N=100–150 mg/liter	Ca=300–500 mg/liter
P= 80–100 mg/liter	Mg= 50 mg/liter
K=150 mg/liter	Fe= 2 mg/liter
B=0.5 mg/liter	Cu=0.05 mg/liter
Mn=0.5 mg/liter	Mo=0.02 mg/liter
Zn=0.1 mg/liter	

The following salts were used in the experiment:

$Ca(NO_3)_2\,4H_2O$	$FeCl_3\,6H_2O$	$CuSO_4\,5H_2O$
KH_2PO_4	$MnSO_4\,7H_2O$	$(NH)_2MoO_4$
$MgSO_4\,7H_2O$	$ZnSO_4\,7H_2O$	$Na_2B_4O\text{-}10\,H_2O$.

The basic nutrient solution was prepared with tap water but the solution of microelements which are diluted less was made up with distilled water. Technical grade salts were used. For our plants a nutrient solution with pH 6-7 appeared favorable. A slight precipitation of phosphorus on the gravel was observed but there was no detrimental effect on growth.

Seven concrete tanks were constructed for gravel culture in small plots in the greenhouse (Fig. 6). Inside of each concrete tank was placed a metal tank

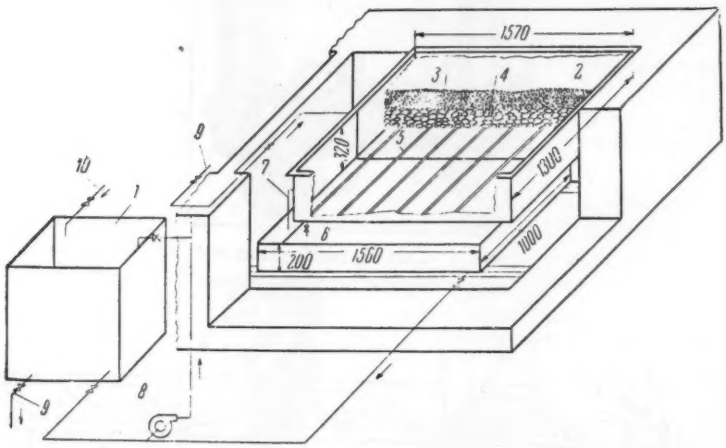

Fig. 7. Sketch of construction of plot for gravel culture of plants. 1) Tank for preparation of fresh solution; 2) gravel chamber; 3) fine gravel (3-7 mm), depth of layer 20 cm; 4) coarse gravel (14-20 mm) for drainage, depth 10 cm; 5) grid of perforated tubes to equalize delivery of solution; 6) tank for effluent solution; 7) overflow of solution from upper to lower tank; 8) pump; 9) drain to sewer for spent solution; 10) tap from water line.

Fig. 8. Tomato plants raised in plots on gravel in the greenhouse.

20 cm deep. On top of this tank was placed another tank 32 cm deep into which coarse gravel (14-20 cm) was poured (10 cm deep) to facilitate drainage. About 20 cm of fine gravel (3-7 mm) was placed on top of the coarse gravel bed (Fig. 7). A "Kama" pump transfered the nutrient solution from the lower tank into the upper tank, from which it flowed downward. A distribution system of perforated tubes laid on the bottom of the upper tank facilitated a uniform supply of fluid. The depth of the solution in the stratum of gravel is determined by outflow openings in the lateral wall two cm below the upper gravel surface. The excess liquid exits through this opening and through a flexible tube into the lower tank. After the entire rooting medium is filled with salt solution a valve is opened and all of the liquid begins to flow through an opening in the bottom of the tank into the tank below; all sides of the tank slope toward this point.

Spent solution flows into the sewer. It is necessary to provide for washing with water the gravel in the tanks containing plants. Fresh nutrient solution is prepared in a special tank from which the solution may be pumped to any plot. Using the described apparatus A. F. Agafonova suceeded in raising tomatoes in gravel culture (Fig. 8). In the future we plan to build beds on the station for gravel culture when the sky is overcast.

SUMMARY

A method is described for growing plants in gravel in pots and small plots. Optional mineral nutrition and good aeration are obtained in this way. Moreover, the root system can be removed from the inert substrate with a minimum of damage to the roots. Gravel culture is also recommended for the investigation of various types of salt tolerance during different plant developmental stages. Tomatoes and seedlings of woody plants were successfully grown for research purposes by the gravel culture method.

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ABSORPTION OF SUBSTANCES BY THE ROOTS AND LEAVES OF CORN

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Non-root nutrition has attracted the attention of many investigators and at present a tremendous number of papers dealing with this important problem have accumulated. By far the most work conducted has involved the non-root application of phosphorous and nitrogen on different crop plants. Both laboratory work and practice show that shoot feeding does not invariably have a beneficial effect. From analysis of the published data it may be concluded that there are two basic reasons for the effectiveness of shoot feeding: 1) how well the compounds applied to the leaves are absorbed by the leaf, are metabolized, and are translocated to other organs and 2) to what extent substances absorbed by the leaf affect uptake of substances by the roots.

Very many investigations have been concerned with the first question. The effect of such factors as temperature and humidity of the air, plant species and age, composition and concentration of compounds applied to the leaf, etc., have been studied with regard to their effect on the absorption and translocation of applied substances.

However, study of the effect of shoot feeding upon absorption of substances through the roots has concerned few workers. In his basic work, Thorne [1-4] showed that certain kinds of nutrient substances applied to the leaves may alter the root absorption of these same substances and also the absorption of other substances. In the experiments of Tueva and Samoilova [5] shoot-applied phosphates retarded the absorption of phosphate by the roots of young squash plants grown for 28 days without phosphorous and then transferred to nutrient solution. In short-term experiments, Matskov and Ikonenko [6], and Ikonenko [7] found that when a 1% solution of urea was applied to tomato leaves the root absorption of P^{32} was appreciably augmented for a ten-day period. Absorption of P^{32} was also accelerated by spraying the leaves with a phosphate solution. Shereverya [8] indicated in experiments with winter wheat that because of soil moisture conditions and soil nutrition shoot feeding does not produce a similar effect upon the uptake of substances by the roots. However, the author did not investigate absorption by roots. He only inferred this by the size of the yield.

The task of this paper was 1) to study the direct link between absorption of nitrogen through the leaves and the absorption of nitrogen and phosphorous through the roots, 2) to ascertain the conditions determining this connection and 3) to study the effect of shoot-applied nitrogen upon the accumulation of protein in the vegetative organs and grain of corn, and upon corn yield.

EXPERIMENTAL METHODS*

The experiments were conducted during 1956-58 in the greenhouse and on experimental plots in the Lenin Hills. The early maturing corn, variety Spasovskaya, was used.

Only nitrogen was used for foliar application. The plants were hand-sprayed with a 4-5% solution of urea with about 0.2% wetting agent OP-10 added. Control plants were sprayed with water plus wetting agent. During the spraying process the soil in the vessels was shielded from contact with droplets of the urea solution.

The plants grown in metal vessels filled with a mixture of eight kilograms of soil and river sand in a ratio of 2 : 1 by weight. Soil moisture was maintained at 70% of field capacity by irrigation of the vessels to the correct weight. In the second experiment ceramic pots containing 5 kg of soil were investigated.

Total nitrogen was determined by the micro-Kjeldahl method, protein according to Barnstein, nitrate according to Grandval and Lesu, phosphorous according to Fiske-Subbaro, sugar according to Bertran, amino acids by paper chromatography with differential color development using isatin according to Boyarkin [9], and types of sugars by paper chromatography [10].

Sap analysis was employed to study absorption of nitrogen and phosphorous by the roots. Sap samples were usually collected for a 24-hour period. From the sap volume and the concentration of nitrogen and phosphorous in it, the amount of these substances in the sap was calculated.

In certain experiments the leaves (4th or 5th from the apex) and culms (internodes without leaves) were selected for analysis. Leaves and culms taken from both treated and control plants were washed off with water before analysis.

*G. V. Faleev assisted in the biochemical analyses.

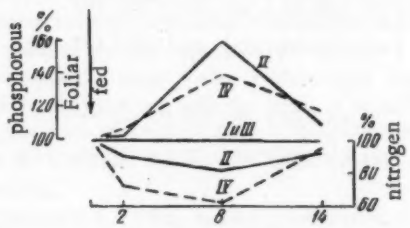
Experiment 1. Scheme of the experiment:

I - Control
II - Foliar fed } average nitrogen level
III - Control
IV - Foliar fed } low nitrogen level

The average-level treatments differed from the low-level treatments in that a normal Hellriegel dose of NH_4NO_3 was added to vessels of treatment I and II prior to sowing and half of a normal dose was added during the eight-leaf stage. The average plant weight in the average-nitrogen treatment (95 g) was twice as great as in the low-nitrogen treatment (46 g).

During the pre-tasseling stage (June 30) a 5% solution of urea was applied to the foliage. Two, eight, and 14 days after the spraying about 12 plants of each treatment were cut off and the exudate collected for 24 hours. The sap volume was measured and sap nitrogen content (Kjeldhal and nitrate) and total phosphorous was determined.

The uptake of phosphorous by the roots was augmented and the uptake of nitrogen was reduced in plants sprayed with urea (Fig. 1). The most prominent effect of spraying upon the uptake of nitrogen and phosphorous by the roots was observed eight days after spraying; the



Nitrogen and phosphorous content of xylem exudate (experiment 1). Percent based on control plants as 100% (treatment I and III), II and IV – plants foliar fed.

difference had nearly disappeared after 14 days. In plants grown with insufficient nitrogen (treatment IV) there was less of an increase in phosphorous absorption when the plants were sprayed but the depression of nitrogen uptake was more strongly expressed compared to plants which had comparatively favorable conditions of nitrogen nutrition (treatment II).

Experiment 2. Plants were sprayed twice with a 4% solution of urea: 1) at the beginning of the tasseling stage (August 10) and 2) during grain-formation at the beginning of the milk stage (September 10). Two and ten days after the spraying, leaf and sap samples were taken. Two days before sampling a normal dose of nitrogen (as NH_4NO_3) was added to the soil (Table 1).

From the data of Table 1, it is evident that during both the entasseling and grain formation stages nitrogen uptake by the roots of sprayed plants is inhibited by the second day after spraying. However, during the tasseling stage ten days after spraying, the uptake of nitrogen by the sprayed plants is still further retarded and during later stages the intake of nitrogen has almost completely recovered 10 days after spraying.

Determinations showed that the nitrogen content of the leaves was higher in the experimental plants than in the controls: 15% in two days and 23% in 10 days after treatment.

Experiment two was conducted at a quite high nitrogen level as might be judged from the high nitrogen concentration in the sap (40-65 mg/100 ml of sap). From this it might be proposed that, because of a high soil nitrogen content, antagonism between nitrogen entering the roots and leaves is stronger here than in experiment one.

The following experiment was carried out in order to show whether there is an effect of the soil nitrogen level upon the degree of retardation of root nitrogen absorption following foliar application of nitrogen.

Experiment 3. The same kind of plants were used here as in the treatments of experiment one. The experiment was set up during the stage before tasseling began as follows.

I - Control
II - Foliar application
III - Root application
IV - Foliar and root application

About the time the experiment began the plants commenced to show a nitrogen deficiency: The lower leaves began to turn yellow and to die. On August 10, half of the plants were sprayed with a 5% urea solution. Leaf and sap samples were taken at three times: 2, 7, and 11 days after spraying. A 0.5 normal dose of nitrogen (as NH_4NO_3) was added to the soil each time two days before sampling the sap. This was done so that, insofar as possible, every time a sample was taken the plants had the same level of soil nitrogen and so that there was never any difference in plant growth because of additions of nitrogen to the soil.

From data on sap nitrogen content (Table 2) it appears that two days after the application of nitrogen to the leaves there is still no marked difference in nitrogen uptake by the roots. After seven days a decrease in nitrogen absorption by the roots was already observed: a 22% reduction in absorption by plants receiving only the foliar application of nitrogen (treatment II) and 36% in plants which received both foliar and soil nitrogen applications (treatment IV). After eleven days have passed, the uptake of nitrogen by the roots of plants which received nitrogen through the leaves returns to the normal level, but in plants which have received nitrogen through both leaves

and the roots (treatment IV) the uptake of nitrogen is retarded still further(by a factor of 2).

Data from this experiment on leaf nitrogen content (Table 2) show that when nitrogen enters the plant only through the leaves (treatment II) the leaf nitrogen content rises 22.5-26% but in foliar + root treatments (treatment IV), the leaf nitrogen content increases only 5-13%.

In order to determine the amino acid content a chromatographic analysis was made of the sap collected eleven days after spraying (experiment 3). The chromatograms showed that in plants sprayed with urea the amount of alanine and glutamic acid in the sap is markedly reduced and, to a lesser degree, so is the amount of threonine, tyrosine, and phenylalanine. An especially abrupt reduction in the amount of the enumerated amino acids occurred in plants which received foliar + soil nitrogen applications (treatment IV), compared to treatment II where the nitrogen was applied to the soil.

The same sap was chromatographed for the determination of sugars. Two ml of sap was placed on each chromatogram. Glucose completely vanished and a certain decrease in the fructose content occurred in the sap of sprayed plants. When the plant absorbed the nitrogen dose through the roots, the fructose content de-

TABLE 1. Nitrogen Content of Corn Leaves and Sap (experiment 2)

Treatment	Two days after foliar application						Ten days after foliar application					
	average dry weight per plant, g	sap nitrogen, mg per 100 ml sap	sap nitrogen, mg per plant	sap nitrogen, mg per plant, % of control	leaf nitrogen % dry weight	leaf nitrogen % dry weight compared to control	average dry weight per plant, g	sap nitrogen, mg per 100 ml sap	sap nitrogen, mg per plant	sap nitrogen, mg per plant, % of control	leaf nitrogen % dry weight	leaf nitrogen % dry weight compared to control
	Beginning of tasseling											
Control	62,5	39.38	3.52	100	2.14	100	68.2	28.50	2.58	100	1.84	100
Foliar-fed	61.7	35.20	2.83	80	2.46	115	75.2	22.50	1.67	65	2.26	123
	Grain forming											
Control	96.0	65.45	1.59	100	—	—	91.4	62.60	1.44	100	—	—
Foliar-fed	111.0	58.00	1.24	78	—	—	114.6	54.80	1.32	92	—	—

TABLE 2. Nitrogen Content of Corn Leaves and Sap (experiment 3)

Treatment	Average dry wt. per plant g	Amount of sap(ml) per plant in 24 hr	Sap nitrogen, mg per 100 ml sap	Sap nitrogen, mg per plant	Sap nitrogen, mg per plant, % of control	Leaf nitrogen, % dry wt. protein	Leaf nitrogen, % dry wt. non-protein	Leaf nitrogen, % dry wt. total	Leaf nitrogen, % dry wt. total, % of control
	Two days after spraying								
I	135.0	33.0	11.74	3.87	100	1.86	0.16	2.02	100
II	127.5	32.5	12.17	3.96	102	2.05	0.49	2.54	126
III	131.25	38.25	19.54	7.47	100	2.24	0.15	2.39	100
IV	149.25	41.25	19.97	8.24	110	2.28	0.41	2.69	113
	Seven days after spraying								
I	127.5	22.6	14.72	3.33	100	1.45	0.15	1.60	100
II	142.0	20.0	13.00	2.60	78	1.73	0.23	1.96	122.5
III	150.0	39.5	25.68	10.14	100	1.92	0.16	2.08	100
IV	147.5	32.0	20.36	6.52	64	2.00	0.18	2.18	105
	Eleven days after spraying								
I	156.0	20.25	6.64	1.34	100	1.32	0.18	1.50	100
II	166.0	18.50	7.76	1.44	107	1.54	0.36	1.90	126
III	160.0	29.75	34.88	10.38	100	1.57	0.11	1.68	100
IV	180.0	35 0	13.56	4.75	46	1.50	0.32	1.82	108

creased but the glucose content changed a little. Sucrose was detected as a barely noticeable spot only in the control. Thus it appears that there is a difference in the use of the different types of sugars: Glucose is expended principally during assimilation of leaf nitrogen but fructose is expended during the assimilation of root nitrogen.

The sugar content of sprayed leaves (Table 3) decreased markedly (20-22%) both in samples taken two and eleven days after spraying (sugar was not determined in samples taken on the seventh day). In treatment III, where the nitrogen was added to the soil, the decrease in sugar content was even more marked; this probably resulted from a greater outflow of the sugars from the root. The increase in sugar content in treatment IV two days after spraying is puzzling.

We have previously observed a decrease in sugar content of wheat leaves sprayed with nitrogen [11, 12] as has Kalinkevich in corn [13].

Thus, it has been shown in short-term experiments that leaf application of nitrogen retards absorption of nitrogen by the roots. We were still interested in the reasons for the observed inhibition of nitrogen uptake. It has been hypothesized that the decrease in nitrogen uptake by the roots of sprayed plants is a result of a deficiency in ammonium acceptors which are used up by combination with ammonium entering the leaves.

In order to test this hypothesis, experiments 4 and 5 were conducted, in which malic or citric acids were applied to the leaves along with the urea. A treatment with sucrose was also included since there are indications in the literature that sucrose added to urea reduces leaf burning.

Experiment 4. Corn, variety Spasovskaya, was used. The experiment was set up during the stage prior to the initiation of tasseling, July 3 (see Table 4 for experimental design). The plants were cut off two days after spraying and the sap collected for 38 hours for nitrogen determination. Six plants were used in each treatment.

Experiment 5. This experiment was set up in the same way as experiment 4, with the exception that ½ a normal dose of nitrogen as NH_4NO_3 was added to the soil in all treatments at the same time that the leaves were sprayed. The plants were sprayed July 11, and after two days the sap was collected for 24 hours. Four replicates were used.

The results of experiments 4 and 5 (Table 4) show that nitrogen absorption by the roots is restored to a marked degree by addition of citric and malic acids to the urea solution. Sucrose did not seem to have any particular effect in these experiments. All leaf-sprayed treatments had a little leaf burn in the form of narrow strips 3-5 mm wide along the edge of the leaves; in treatment IV the strips were somewhat wider than in treatment II.

Experiment 6. In the field the effect of early and late spraying of foliage with urea were studied in relation to protein accumulation and the yield of corn, variety Spasovskaya. A 5% solution of urea was applied to the foliage at two times: during initiation of tasseling (July 14-30) and during the silking–grain formation stage (September 1-11). During each of these times three foliage applications were made at intervals of 5-7 days. The experiment was evaluated on September 26 during the milk stage. In addition, five days after the early application (August 4), samples were taken in order to trace changes in nitrogen in the plant over short periods of time after spraying (Table 6). Each plot had 18 plants and was replicated twice.

The data in Table 5 show that spraying the shoot with nitrogen later (during the silking–grain filling period) was more effective for increasing protein content than spray application of nitrogen during an earlier period (at the beginning of tasseling). The total nitrogen content with the late application (treatment III) rose in the leaves 32% and in the culms 41%. With early spray application (treatment II) the increase in nitrogen content was respectively 8 and 25%. However, in plants of this treatment in the sample of August 4, taken 5 days after the early application, the leaf nitrogen content rose appreciably (37%) while the nitrogen content of the culms remained unchanged. So far as the effect of foliage feeding upon yield is concerned it can only be noted that shoot application during early stages influences the formation of ear primordia, somewhat increasing the number

TABLE 3. Amount of Water-Soluble Sugars in Leaves (experiment 3)

Treatment	Two days after spraying		Eleven days after spraying	
	% of dry wt.	% of control	% of dry wt.	% of control
I. Control	4.60	100	8.11	100
II. Foliar application	3.59	78	6.52	80
III. Root application	2.47	54	5.78	71
IV. Foliar and root application	5.74	125	6.34	78

of ears per plant. However, we can say that the weight of ears per plant and the weight of the entire plant is not increased by either early or late foliage application of nitrogen.

One of the fundamental reasons for the greater effectiveness of late foliage applications could well be the fact that, as noted in experiment 2, the late application retards the absorption of nitrogen by the roots less than does foliage application at an earlier date.

In other field experiments the following increase in the nitrogen content of corn which had a late foliage application of nitrogen were noted: early-maturing variety Spasovskaya leaves 23.5%, culms 30%, grain 14%; late-maturing variety Sterling leaves 16%, culms 25%.

DISCUSSION OF RESULTS

The effect of shoot application of nitrogen upon absorption of substances by the roots may be divided depending upon conditions into 1) a direct effect, which appears rapidly before morphological changes occur, and 2) an indirect effect, when the effect has been complicated by the growth processes, particularly by an increase in root absorbing surface. In one experiment the spraying of corn leaves with a solution of urea retarded nitrogen absorption by the roots (experiments 1-5) and augmented phosphorous absorption (experiment 1). This effect can be considered as direct and simple since it was detected by the second day after spraying; in samples taken after 5-7 days the effect was augmented and after 11-14 days it was attenuating although under certain conditions even after 14 days the inhibition of nitrogen absorption had not diminished.

The reduction in nitrogen absorption by roots of plants sprayed with a urea solution is probably a result of a certain derangement in the circulation of organic substances in the plant. The existence of a circulation of substances in the plant has been established by the work of Kursanov et al. [14, 15, 16]. Normally, the products of photosynthesis are translocated from the leaves to the roots where they go through glycolysis and Krebs cycle and are transformed into keto acids. These acids are combined with ammonium taken up from the soil to produce amino acids and amides, part of which are translocated to the shoot and part are utilized in the growth processes of the roots themselves. It has also been shown [17] that the formation of carbohydrate acceptors in the roots is closely linked to the absorption function of the roots: The flow of assimilates from the

TABLE 4. Root Absorption of Nitrogen by Plants Sprayed with a Urea Solution Containing Citric and Malic Acids

Treatments	Experiment 4				Experiment 5			
	sap per plant per day, ml	nitrogen content, mg			sap per plant per day, ml	nitrogen content, mg		
		per 100 ml sap	per plant	per plant, % of control		per 100 ml sap	per plant	per plant, % of control
Control	21.50	10.42	2.24	100	37.5	30.26	11.35	100
Urea 8%	22.16	4.72	1.05	47	35.0	21.63	7.57	67
Urea 8%+Citric acid 0.5M	21.83	7.68	1.67	75	33.75	30.14	10.17	90
Urea 8%+Malic acid 0,5M	21.33	7.82	1.67	75	36.25	25.02	9.07	80
Urea 8%+sucrose 0,25M	19.67	6.08	1.20	54	35.0	22.03	7.7	68

TABLE 5. Effect of Spraying Maize Shoots with Urea in Relation to Nitrogen Content and Yield

Treatment	Sampled August 4 – after early spraying				Sampled September 26 – after late spraying						
	total nitrogen				total nitrogen				ears/plant	wt. ears/plant	wt. plant with ears
	leaves		culms		leaves		culms				
	% of dry wt.	% of control	% of dry wt.	% of control	% of dry wt.	% of control	% of dry wt.	% of control			
I. Control	1.79	100	0.80	100	1.64	100	0.44	100	1.0	119	355
II. Early spraying	2.45	137	0.82	102.5	1.77	108	0.55	125	1.3	122	372
III. Late spraying	–	–	–	–	2.16	132	0.62	141	1.1	119	363

leaves to the roots and the rate of their conversion is increased by absorption of the ammonium by the roots.

When nitrogen is applied to the leaves this relationship in the circulation of substances in the plant is altered. The assimilates are used by the leaves themselves for ammonium acceptors. The formation of amino acids in leaves was observed by Khokhryakova [18] when the leaves were infiltrated with mineral nitrogen. Despite the fact that spraying the leaves with nitrogen elicits an increase in the rate of photosynthesis [19, 20], the leaves of plants sprayed with urea have appreciably less sugar than normal leaves. It is evident that the flow of assimilates from leaves sprayed with nitrogen and into the roots is reduced and that this is the reason for a reduction in assimilation of ammonium from the soil by roots of spray-treated plants. The addition of citric or malic acid to the urea solution to a marked degree reestablishes the absorption of nitrogen by the roots. This may indicate that the initial assimilation of nitrogen in the leaves just as in the roots occurs in conjunction with the acids of the TCA cycle.

Probably, the effectiveness of foliage application of nitrogen will be determined by how marked will be the changes in the circulation of organic substances in the plant assimilating nitrogen in the leaves and, primarily, to what extent the supply to the roots of sugars from which carbohydrate acceptors of ammonium are formed within the roots is interrupted. Therefore, supplying the plants with sugar at the time when nitrogen is applied to the foliage probably ought to be considered one of the fundamental internal factors conditioning the effectiveness of foliage application of nitrogen. In other words, the effectiveness of the application of nitrogen to the shoot will, on the one hand, depend upon the relative amount of nitrogen absorbed by the leaves, and, on the other hand, upon the amount of the restriction in nitrogen absorption by the roots produced by foliage absorption of nitrogen.

SUMMARY

Experiments conducted using mature corn plants grown in vessels and on small plots showed that spraying the leaves with a 4-5% solution of urea decreased the uptake of nitrogen through the roots.. This effect of foliar application of nitrogen continued, depending on the conditions, for 1-3 weeks and was due to a deficiency of ammonia acceptors which were consumed in the fixation of ammonia absorbed by the leaves.

The protein content of the vegetative organs and seeds of corn was appreciably augmented when the plants were sprayed with the urea solution. Late foliar application was more effective after formation of the panicle. After three sprayings the protein content in the leaves increased by 16-32%, in the stems by 25-41%, and in the grain by 8-12% compared with the control.

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AMINO ACID COMPOSITION OF VARIOUS MATURITY WATERMELON SEED GLOBULINS

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The problem of changes in the composition and properties of protein in the life cycle of plants should today be considered as one of the most important subjects in the study of plant protein metabolism. Unfortunately, the number of investigations dedicated to this problem is still quite small.

As to changes in the amino acid composition of seed proteins during the ripening process, we have at our disposal the data of M. Kostrubin for wheat, of P. Agatov on rye, and of E. Bezinger for flax [1]. The changes in the amino acid composition of proteins in ripening seeds, as observed by these authors, are of great and varied interest.

The objective of the present investigation was to study the amino acid composition of globulins in watermelon seeds during maturing on the vine, as well as during the post-harvest ripening period.

We chose for our study the seed of two kinds of watermelon, which differ greatly as to their maturation period as well as other biological, biochemical and economical properties. Both types belong to the species Citrullus edulis Pang. The Stokes variety has small seeds and is an early one; the Melitopol variety No. 142 has large seeds and is a late one.

METHODS

In order to obtain a uniform seed material, the seeds were collected from watermelons grown on plots of the experimental cultures on the instruction farm "Roshcha" at the I. V. Michurin Fruit-Vegetable Institute, where identical cultural methods were practiced. The seeds for the present investigation were collected in 1949. The watermelons for seed collection, of various degrees of maturity, were selected on the basis of being derived from germs of the same age and coming from branches of the same order. The degree of ripeness of the fruit as well as the exterior condition of the seed itself were taken in consideration in setting the collection date for seeds of various maturity (Table 1).

The unripe seeds (I) were collected from unripe fruit. The not fully ripe seeds (II) were collected from fruit slightly before they would have reached consumers' ripeness. The seeds of the third stage or ripeness (III) were collected from fruit having reached the consumers' acceptance stage. The seeds of the fourth and fifth groups were obtained from fruit removed from the vines on the day of the third group's harvest, but here the watermelons were allowed to continue to ripen for some time, and the fourth collection was made at the stage of biological ripeness of the fruit (IV). The fifth collection of seeds was made from fruit with the first signs of pericarp decomposition (V).

The thus collected seeds were washed with water and dried on sheets of filter paper at room temperature until air-dry. In every case, the seeds were being analyzed only a month after their collection. During this interval the seeds reached air-dry state and constant weight, as established on controls. Only the cotyledons with the germ were used in this study, the hull being rejected.

The defatted flour of watermelon seed was used as the material for globulin extraction. The defatting was invariably conducted by cold leaching on standing with ethyl ether dehydrated by means of anhydrous sodium sulfate.

Fractionating extraction of the proteins by water, by solutions of sodium chloride and sodium hydroxide, and by alcohol showed that the predominant protein of watermelon seeds is a globulin soluble in a 10% solution of sodium chloride, this being true for all stages of seed maturity under investigation [2].

The globulin was precipitated from the salt solution by dialysis. The precipitate, the globulin, was again dissolved in a 10% solution of sodium chloride, and again dialyzed. After this the protein was filtered off and dried by means of increasing concentrations of alcohol, and subsequently by ether. The dried globulin was in the form of a snow-white powder of high dusting capacity.

The hydrolysis of the globulin materials, the separation of the products of hydrolysis and the determination of amino acids was conducted by the method of Kizel' [3]. Histidine was determined colorimetrically by Pauli's method; arginine, by alkali decomposition according to Plimmer; lysine, from the nitrogen in the phosphotungstic acid precipitate; tyrosine, colorimetrically, with Millon's reagent according to Thomas; proline, by difference between the total nitrogen of the hydrolyzate fraction and its amino nitrogen, obtained in a one-half hour determination in Van Slyke's apparatus; tryptophane colorimetrically, by Fürth's method in a weighed sample of non-hydrolyzed protein. The quantative determination of dicarboxylic amino acids (without their separation into glutamic and aspartic acids) was conducted chromato-

TABLE 1. Dates of Seed Collection

Variety	Maturing on Vine			Ripening after harvest	Decomposition of pericarp is beginning
	I	II	III	IV	V
Stokes	7/29	8/5	8/12	10/24	12/5
Melitopol No. 142	8/19	8/27	9/6	12/3	2/13 (1950)

TABLE 2. Various Forms of Nitrogen and Some Amino Acids in Globulins from Watermelon Seeds of Various Stages of Maturity (in % of dry de-ashed protein)

Kind of Nitrogen and Amino Acid	The Stokes variety					Melitopol variety No. 142				
	maturing on vine			ripening after harvest	decomp. of pericarp starts	maturing on vine			ripening after harvest	decomp. of pericarp starts
	I	II	III	IV	V	I	II	III	IV	V
Kind of nitrogen:										
total nitrogen	18.35	18.47	18.53	18.87	18.48	18.38	18.56	18.71	19.01	18.57
monoamino N + lysine	7.62	7.78	7.92	8.02	7.80	8.00	8.04	8.22	8.48	8.01
alpha amino nitrogen	5.92	5.86	5.87	6.03	5.93	6.01	5.92	6.03	6.17	5.98
alpha + epsilon amino nitrogen	6.87	6.89	6.91	7.05	6.89	7.14	7.04	7.11	7.42	7.09
cyclic nitrogen	0.75	0.89	1,01	0.97	0.91	0.86	1.00	1.11	1.06	0.95
insoluble huminic nitrogen	1.03	0.87	0.62	0.51	0.97	0.95	0.72	0.49	0.45	0.84
Amino acids:										
arginine	11.30	13.72	15.57	16.01	15.05	13.76	16.05	18.48	19.42	17.92
histidine	1.88	2.18	2.42	2.50	2.53	1.12	1.42	1.74	1.88	1.99
lysine	5.47	6.00	6.05	6.16	6.02	6.33	6.94	7.02	7.30	7.12
tyrosine	2.47	2.46	2.51	2.48	2.01	2.49	2.52	2.61	2.62	1.87
aminodicarboxylic acids	29.53	30.17	24.71	21.30	18.12	26.91	25.73	22.23	18.92	14.78
proline	6.19	7.31	8.25	8.01	7.47	7.07	8.24	9.12	8.72	7.60
tryptophane	2.02	2.30	2.47	2.73	2.33	1.38	1.61	1.75	2.39	1.92

graphically [4]. To evaluate the degree of purity of the isolated globulin samples, determinations of their ash and total nitrogen were also performed.

In conducting the study of the amino acid composition of globulins from watermelon seeds of different maturity, the main attention was focused on the ones with dependable methods for their quantitative estimation. Duplicate hydrolytic procedures on separate weighed out samples of the same protein materials invariably gave for all of the amino acids well-matching results.

RESULTS

Table 2 gives the results of determinations on hydrolyzates of globulins isolated from watermelons at various stages of their development.

It can be seen from the reported analytical material that the protein in the immature watermelon seeds, the globulin, seems to be already fully formed in respect to the total nitrogen and amino acid composition. But one should also take note that in the course of the ripening process of the seeds, there take place changes in the composition of the deposited globulins, which are beyond a potential analytical error.

Therefore, while there is only an insignificant increase in the total nitrogen content in the globulins of the maturing seeds, there take place changes in the contents of certain amino acids, indicating an evident rebuilding of the proteins in accordance with the general trend in the synthetic processes in the seeds during this period [2, 5].

These changes consist of an increase in arginine, histidine, lysine, tryptophane and proline, and a significant lowering of dicarboxylic amino acids, while tyrosine remains stationary. One gets the impression that the increase in the enumerated amino acids is taking place at the expense of the simultaneously decreasing aspartic and glutamic acids. These amino acids evidently serve as a nitrogen reserve, at the expense of which other amino acids are being formed. Some time ago, Dubrovskaya [6] called attention to the greater lability of dicarboxylic amino acid in her study of the composition of wheat proteins from frostbitten as well as normally maturing wheat grain.

It is very important to stress here that the changes observed by us in the globulins of maturing watermelon seeds are valid also in the case where the watermelon is

TABLE 3. The Amino Acid Contents (in %) in the Globulins of Watermelon Seeds of Biological Maturity (IV) and in Seeds Allowed to become Overripe in the Fruit (V)

Amino acids	Stokes variety			Melitopol variety No. 142		
	maturity		changes	maturity		changes
	IV	V		IV	V	
Arginine	16.01	15.05	—6.0	19.42	17.92	—7.7
Histidine	2.50	2.53	+1.2	1.88	1.99	+5.9
Lysine	6.16	6.02	—2.3	7.30	7.12	—2.6
Tyrosine	2.48	2.01	—19.0	2.62	1.87	—28.6
Tryptophane	2.73	2.33	—14.6	2.39	1.92	—19.6
Dicarboxylic amino acids	21.30	18.12	—14.9	18.92	14.78	—21.9
Proline	8.01	7.47	—6.8	8.72	7.60	—12.8

TABLE 4. Varietal Differences in the Amino Acid Composition of Globulins from Watermelon Seed (IVth grade of maturity)

Amino acids	Amino acid content (in % of protein)		Maximal varietal difference (%)
	Stokes	Melitopol No. 142	
Arginine	16.01	19.42	21.3
Histidine	2.50	1.88	33.0
Lysine	6.16	7.30	18.5
Tyrosine	2.48	2.62	5.6
Tryptophane	2.73	2.39	14.2
Dicarboxylic amino acids	21.30	18.92	12.5
Proline	8.01	8.72	8.9

ripening off the vine (grade IV of maturity). Thus, ripening after harvest is, from the point of view of protein formation, nothing else than a continuation of the physiological process of maturation. The changes in amino acid composition during the ripening of seeds are also an indication of the fact that the amino acids subject to modification are the ones which carry a special function in the maturing seed. The differences observed in relation to the stages of maturity of seeds, which are beyond analytical error, are a definite indication of qualitative changes in the proteins of watermelon seeds during ripening, and of a continuous change in the proteins as related to the age of the seeds.

It is proper to refer to our earlier paper [7] for findings on changes in the amino acid composition of watermelon leaf proteins with the age of the plant.

The list of amino acids reported in this paper does not by any means give a complete picture of protein (globulin) synthesis in the process of watermelon seed ripening. To achieve this, the number of amino acids accounted for in the protein hydrolyzates should be increased to the limit. Only in such a case would it be possible to obtain a true picture, in all its scope, of the process of protein synthesis during maturation of seeds.

Where the seeds have been allowed to become overripe within the fruit (stage V of maturity), the character of amino acid changes in the globulins is taking another direction. The magnitude of these changes is shown in Table 3.

It is plainly evident from Table 3 that in the globulins of seeds which are held over in the fruit to the point of symptoms of decay of the pericarp, there begins a noticeable lowering in arginine, proline, aminodicarboxylic acids, tyrosine, and tryptophane. Especially noticeable at this period is the lowering in tryptophane, tyrosine, and aminodicarboxylic acids. The amount of lysine and histidine remains here practically unchanged. The data of Table 3 demonstrate a different stability of individual amino acids during autolysis, which at this time is taking place in watermelon seeds.

We would like to point out also that we were able to observe in the seeds of stage V ripeness a lowering in the dry substance and an increase in water content, a lowering in oil with a simultaneous change in its physicochemical properties, such as a lower iodine number and a higher acid number [5]. There was also noticed in these seeds an increase in nonprotein nitrogen with a simultaneous decrease in protein nitrogen [2]. All these data indicate definitely that in the watermelon seed at maturity stage V there takes place a trend in the direction of a predominance of hydrolytic processes over synthetic ones, while all of the preceding phases of seed ripeness (I, II, III, and IV) are characterized by a predominance of synthetic processes over hydrolytic ones [2, 5].

These changes in the seeds of grade V maturity result in a lowered vitality and decreased quality as seeds [8]. We were also able to show in the same paper that seeds of grade IV maturity are most viable and of highest quality.

As to the amino acid composition of globulins of different varieties, one can state that through all the ripening stages of watermelon seeds the globulins of the late Melitopol variety 142 contain more arginine, lysine, and proline, and less histidine, trypotophane, and dicarboxylic amino acids than the globulins of the early Stokes variety. As to tyrosine, there is no essential difference. Table 4 shows the varietal differences in the amino acid composition of the globulins in seeds of stage IV ripeness.

The high arginine content, especially in the seed globulin of the Melitopol 142 variety, can not escape notice. The comparatively high percentage of proline leads one to speculate about its potential secondary formation from citrulline. Data in the literature [9] indicate the possibility of proline formation from citrul-

line during acid hydrolysis of protein, and of ornithine in alkaline hydrolysis.

The high percentage of nitrogen in the watermelon seed globulins and the insignificant amount of insoluble huminic substances (see Table 2) indicate a sufficiently high purity of our isolated protein materials.

SUMMARY

The reserve protein, globulin, from unripe watermelon seeds resembles greatly the globulin of ripe seeds in total nitrogen content and amino acid composition. Besides, the globulins of ripening seeds undergo changes in the content of certain amino acids, indicating a definite rebuilding of the protein. These changes in the amino acid composition of globulins of ripening watermelon seeds continue their trend also during subsequent ripening of the fruit after removal from the vine. These changes in the globulins continue until the seeds have reached the stage of biological maturity.

The globulins of seeds which were allowed to become overripe while in the fruit exhibit a character of amino acid changes in a direction quite opposite to the ones observed during ripening on or off the vine.

The amino acid composition of globulins from seeds of two watermelon varieties of different vegetative periods exhibit essential differences during the entire periods under study.

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BRIEF COMMUNICATIONS *

THE EFFECT OF GIBBERELLIN ON THE GROWTH AND YIELD OF HEMP AND TOBACCO

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The clearest feature in the action of gibberellin on plants is the ability to stimulate the growth of above-ground parts. It has been established that under the effect of gibberellin, the formation and growth of the plant stem and its lateral shoots and leaves are stimulated and the growth of parthenocarpic fruits is increased [1-6].

In connection with this it would be very interesting to study the effect of gibberellin on technical crops and, in particular, on hemp, the yield of which is connected with an increase in the length of the stem, and on tobacco, the yield of which depends on an increase in the number and weight of the leaves. We carried out detailed experiments in the summer of 1959 in the greenhouse, Exhibition of Achievements in the National Agriculture of the USSR, in which southern Chuiska hemp (Cannabis sativa) and Mamont tobacco (Nicotina tabacum) were used as objects in the experiment. The experiments were carried out in soil in Mitcherlikh vegetation containers of 5 kg to which a complete mineral fertilizer was applied prior to planting.

Hemp seeds were planted in the containers on May 6 and the shoots appeared on May 11; tobacco seeds were planted in pica dishes on March 24, shoots appeared on March 31, cuttings were made on April 25 and transplanting into vegetation containers was done during the two-leaf phase on June 1. At the beginning, eight hemp plants were placed in each container and at the end of the experiment this number was brought down to four and even to two; in the case of tobacco, there was only one plant in a container from the beginning to the end of the experiment. The experiments were started on June 19 and finished on October 3.

Half of the hemp and tobacco plants (three containers) was subjected to treatment with gibberellin and the other half was left as a control. The experimental plants of hemp and tobacco were sprayed with weak solutions of gibberellin on five dates: June 19 and 26, July 3 and 10, and August 7; the tobacco plants received an additional spraying on September 7. The concentrations of the solutions were 0.001% for the first spraying, 0.002% for the second spraying, and 0.01% for the following sprayings. A wetting agent (OP-7 or OP-10) in a concentration of 0.05% was added to the solution for spraying. Control plants were sprayed on the same dates, with water containing the wetting agent. Prior to the beginning of the experiment on June 19, the hemp plants were approximately 80 cm tall and the tobacco plants were 20 cm tall and had four to five leaves.

The gibberellin treatment had a very marked effect on the plants: The growth of the stems of the plants was noticeably increased, and the experimental plants significantly outdistanced the control plants in growth with the passage of time (Table 1).

The increased growth of the treated plants took place partially by an increased length of the internodes and partially by an increase in the number of leaves.

TABLE 1. The Effect of Gibberellin on the Growth of Hemp and Tobacco Plants (in cm)

Plant	Experimental variant	July 6	July 13	July 22	July 29	August 3	August 10	August 19	September 3
Chuiska hemp	Control	111	130	140	160	165	170	185	195
	Gibberellin	182	232	300	350	365	405	445	485
Mamont tobacco	Control	25	30	45	55	62	70	80	90
	Gibberellin	35	67	110	120	135	160	175	215

In the first period of growth, the leaves of the experimental plants were of significantly greater dimensions; the leaves which appeared later were little different from the control leaves of the hemp; however, the tobacco leaves were longer, but not wider, than the control leaves. The experimental tobacco plants were differentiated from the control plants by the lighter color of the leaves; the lightening of the leaves of the hemp plants was observed to a lesser degree.

* "Brief Communications" are devoted to the results of tests of gibberellin on various agricultural plants. These tests were organized in 1959 on the initiative of the Growth and Development Laboratory, K. A. Timiryazev Institute of Plant Physiology, USSR Academy of Sciences. Besides this, the editor has considered it proper to insert the communication of V. I. Razumov dealing with the use of gibberellin for accelerating the flowering of short-day plants.

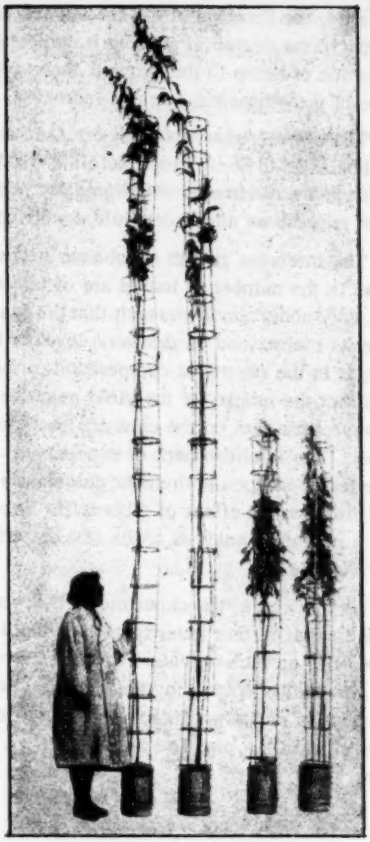

Fig. 1. The effect of gibberellin on the growth and development of southern Chuiska hemp. Left, two containers with plants sprayed five times with gibberellin in concentrations of 0.001-0.01%; right, control plants (Photo September 28, 1959).

Fig. 2. The effect of gibberellin on the growth and development of Mamont tobacco. Left, two plants sprayed six times with gibberellin in concentrations of 0.001-0.01%; right, control plants (Photo September 27, 1959).

TABLE 2. The Effect of Gibberellin on the Growth, Development, and Yield of Plants of Hemp and Tobacco

Plant	Experimental variant	Phase of development	Ht. of plants, cm	No. of leaves on one plant	Green weight of one plant	
					hemp	parent tissue
Chuiska hemp	Control	Phase of seed formation	205	–	74	138
	Gibberellin	Phase of partially formed seeds	510	–	186	289
Mamont tobacco	Control	Vegetative growth	100	22	340	
	Gibberellin	Vegetative growth	250	43	740	

In connection with the sharp elongation of the stems and in order to guarantee normal growth, we carried out a systematic feeding of the plants with nutrient substances.

The increased growth of the experimental hemp plants resulted in flowering beginning later than in controls; control plants started on August 25 and experimental plants on September 2. Toward the end of the experiment the hemp plants were not able to form ripe seeds and were in the phase of seed ripening (in the green phase), while the control plants were somewhat riper than the plants treated with gibberellin. The tobacco plants, both the experimental and control plants, did not form buds and flowers and remained in the phase of vegetative growth. The plants were collected and the

weight of their above-ground mass was calculated at the end of the experiment on October 3, after which measurements and photographs were made of them. The data thus obtained are presented in Table 2 and in Figs. 1 and 2.

The data in Table 2 and Figs. 1 and 2 clearly show that the length of the plants is increased by two-and-a-half times under the effect of gibberellin, and the weight of the green mass is increased by one-and-a-half to two times; the number of leaves is doubled for tobacco.

The vigorous growth of the hemp plants which takes place under the effect of gibberellin obviously is of interest in the practice of horticulture. However, two questions arise here: To what degree are hemp plants reaching heights greater than 5 m resistant to breakage, and what is the output and technical quality of the hemp obtained from plants treated with gibberellin?

The answer to the first question can be given only by experiments established under field conditions with various densities of growth and under various conditions of water and mineral nutrition. The answer to the second question is given to a certain extent by data from laboratory investigations of the hemp stems, which were carried out in the Central Scientific Research Institute of Flax and Bast Fibers (TsNIILV).

Table 3 shows that when hemp plants are treated with gibberellin, the technical length of the stem (that is, the length of the stem from the clump to the beginning of branching at the top of the stem) is significantly increased, the duration of the soak is decreased, homogeneity in the degree of soaking is achieved, the concentration of hemp in the stems is increased, and the staple of the elemental hemp is increased.

These preliminary data suggest that the application of gibberellin to hemp crops can show good results and that further experiments with hemp are necessary, both in the greenhouse and under field conditions.

The intensive growth of tobacco stems and the increase in the number of leaves are of interest for this crop, only under conditions such that the quality of the leaves is maintained on the same level or in any case, changes in the chemical composition of the leaves do not affect the quality of the yield negatively. We do not have such data on the chemical composition of the leaves. The establishment of experiments on different varieties of tobacco, both under greenhouse and field conditions, on the effect of gibberellin in connection with a chemical analysis of the raw material would be very expedient.

On the whole, the experiments that were carried out show that further investigation of the action of gibberellin on such technical crops as hemp and tobacco, with the goal of increasing their yields and deciding the question of the possibility of the application of gibberellin in the practice of horticulture, has a bright future.

TABLE 3. The Effect of Gibberellin on the Morphological and Technical Characteristics of Chuiska Hemp

Indicator	Control			Gibberellin		
	parent tissue	hemp	sample as a whole	parent tissue	hemp	sample as a whole
Number of stems (each)	6	4	10	2	3	5
Average technical length (cm)	178	182	180	371	447	416
Average diameter of stem in the middle (cm)	6.7	6.4	6.6	11.0	8.3	9.4
Diameter in the clumps (cm)	8.7	8.4	8.6	14.5	12.4	13.2
Impurity from leaves and flowers (% of total weight of the plants)	34.3	15.1	26.6	5.5	4.8	5.0
Output of hemp (% of stem weight)	8.0	10.1	8.8	10.8	12.0	11.5
Soaking time (hours)	144	96	–	70	70	–
Average length of the hemp fibers (mm)	–	–	9.4	–	–	14.8
Weight of staple (%)						
Up to 15 mm	–	–	65.6	–	–	54.4
15-50 mm	–	–	34.4	–	–	45.6
Staple count (%)						
Up to 15 mm	–	–	77.9	–	–	50.8
Over 15 mm	–	–	22.1	–	–	40.2

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THE EFFECT OF GIBBERELLIN ON THE GROWTH OF YOUNG TEA SHOOTS

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Gibberellin is distinguished by its high physiological activity. In stimulating the processes of growth, it shows a regulating effect on the development of plants [1-3] and on the yield of green mass of various agricultural crops [4, 5].

The study of the effect of gibberellin in the activity of the growth processes of the tea bush is of significant interest because the yield of this crop is determined by the output of the green leaf, which is an immediate product of the growth processes. With this as a goal, we carried out special investigations under field conditions. At the end of September, 1959, on the tea leaf plantation of the Lenkoransk Section of the Azerbaijan Scientific Research Institute of Horticulture, Viticulture, and Subtropical Crops, a level trellis was selected where tea bushes could be distinguished from each other by their external features. After making the sixth collection from the bushes, such a trellis was divided into eight parts of two linear meters each. Four of them served as controls and the remaining four as experimental plots. The experimental and control plots were alternated, thus obtaining four repetitions. The tea bushes on the experimental plots were sprayed with a gibberellin solution with a concentration of 200 mg/liter on the following dates: September 25, 28, and 29, and October 2 and 5, 1959. 300 ml of the solution was applied in each spraying on all four experimental plots (8 linear meters).

On October 7 and 8, the transition of the axillary buds from the period of concealed growth into visible growth was observed and the spraying was stopped. Further observations showed a positive action of gibberellin on the growth of the young tea shoots. In spite of the cold (average October temperature in 1959 was 6° below normal), the axillary shoots of the tea bushes treated with gibberellin vegetated normally but the growth on the control bushes was very poor.

The effect of gibberellin on the growth of shoots is characterized by the data presented in the table.

The data in the table show that young shoots of tea bushes treated with a solution of gibberellin grow during the period of October and the beginning of November, while only individual shoots, changing into a period of visible growth are found on control bushes. It is interesting that gibberellin removes the effect of autumn cold, which suppresses the growth processes. The average growth per shoot was 45.1 mm for 20 days (from October 2 to November 11) and an average of 0.8 leaves appeared on each shoot during this same period.

It must be noted that gibberellin showed a stimulating action on the growth not only of the upper axillary shoots; lower shoots also started to grow under its effect, as the picture so well demonstrates.

In order to characterize the degree of the appearance of young shoots on tea bushes, we counted the number of one-, two-, and three-leaved shoots on the trellis plots, the area of which was 1000 cm^2, on November 11, 1959. The average data for the four repetitions showed the following.

On the surface of the indicated area of tea bushes treated with gibberellin, 12.7 three-leaved, 16.3 two-leaved and 14.7 one-leaved shoots were counted from a total of 43.7 shoots, which suggests normal vegetation of the plant, while not a single shoot was found on the control.

It must be noted that in dividing the trellis into plots, sometimes the border fell in the middle of a bush; half of the bush was placed in the experimental plot and the other half in the control plot. In such cases the

Indicators of Growth of Young Shoots of the Tea Bush (average per shoot)

Experimental variant	October 21		November 11	
	length, mm	number of leaves	length, mm	number of leaves
Control	–	–	11.8	0.2*
Gibberellin	29.8	2.2	74.9	3.0

*Individual shoots with one undeveloped leaf were found on control bushes.

Left – untreated shoot; right – shoot treated with gibberellin.

growth of young shoots was observed on the parts of the bush treated with gibberellin, while the growth processes were the same on the untreated (control) portion as on the remaining control bushes.

We made a collection of green leaf on the control plot on November 11, the yield of which was as follows:

Experimental variant	Per plot, 2.8 m^2	kg /hectare
Control	0.66	2.35
Gibberellin	42.8	152.80

Toward the middle of November a yield of green leaf deserving of attention was obtained from the tea bushes treated with gibberellin.

We extend our thanks to Professor M. Kh. Chailakhyan for sending a preparation of gibberellic acid and for his aid in consultations.

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THE EFFECT OF GIBBERELLIC ACID ON DIFFERENT VARIETIES OF GRAPE

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Gibberellin, a substance possessing an extremely high physiological activity, is being given much attention in scientific literature. Many articles have appeared in Soviet scientific literature in which the results of investigations connected with an explanation of the nature of the effect of gibberellin on the growth and development of various plants have been briefly stated [1-4].

The grape plant is undoubtedly one of the primary objects of a number having specific interest from the point of view of the character of the response to the action of gibberellin.

Foreign authors have established [5, 6] that treatment with solutions of gibberellin of the flowers of seedless varieties of grape causes a very significant increase in the dimensions of the fruit, sometimes five-fold, and consequently, a great increase in the yield. This method, according to Viver [5], can supplant completely one of the most laborious processes in viticulture–the cutting of shoots–without any loss in effectiveness. The grape clusters are looser under the effect of gibberellic acid, and this decreases disease [4].

There are contradictory reports in the literature with respect to the effect of treatment of seed grape varieties with solutions of gibberellin. Thus, Viver [5] says the varieties of grape having seeds in the fruits are not responsive to treatment with gibberellin. According to the author, this can be explained by the fact that natural substances similar to gibberellin are formed in the seeds and help in the normal development of the fruit. The size of the fruit for such varieties is not increased after treatment with gibberellic acid. There are, however, indications [4] that approximately the same results have been obtained with varieties of grape having seeds as with seedless varieties.

The results that have been presented of experiments using solutions of gibberellic acid in viticulture led us to test the action of gibberellin on grape in the conditions of the Crimea. We planned to carry out preliminary observations on varieties differing in biological characteristics, because of the limitation of available knowledge of the effect of gibberellin on the grape plant. Seedless varieties of grape, such as Kishmish (various), Askeri, and varieties having normal seeds were treated with solutions of gibberellin. Two types of varieties were included in the latter group: bisexual varieties not demanding specific pollination with the pollen of other varieties (Royal Vineyard and Al'burla), and varieties with a functionally-female-type flower having sterile pollen, and as a result requiring pollination by pollen of other varieties for normal development (Nimrang and Pukhlyakovskii).

The so-called parthenocarpic fruits, or seedless grapes, which form without fertilization, are usually of a very small size, significantly smaller than the normal fruits of these varieties with normal seeds which develop after pollination. On examination of these varieties, it was proposed to establish the role of the seeds in the reaction of grape to treatment with solutions of gibberellic acid and the effect of gibberellic acid on the formation of grapes and development of parthenocarpic fruits.

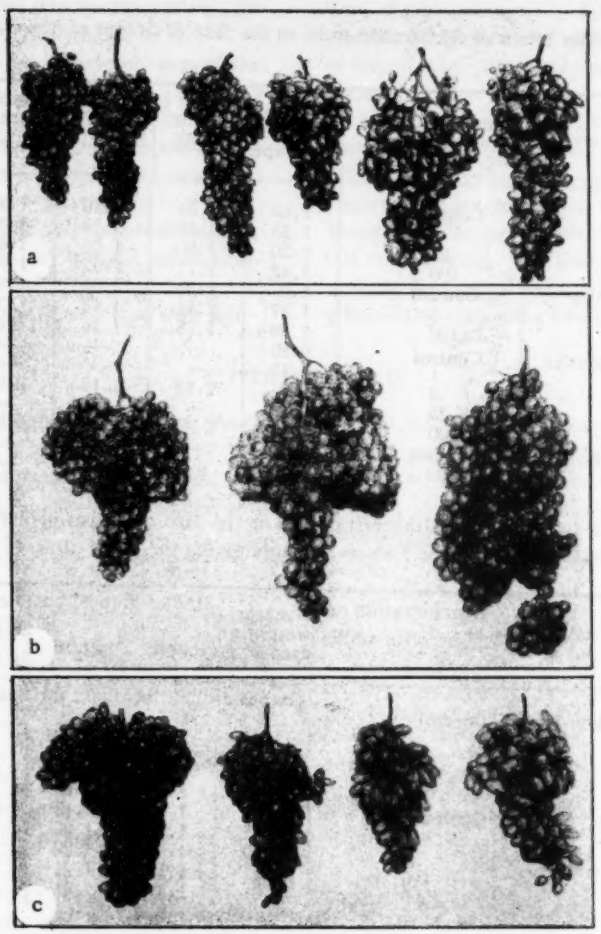
Mainly we applied gibberellic acid which was prepared in the Biological Section of the Czechoslovakian Academy of Sciences* in the experiment. Besides this, we applied gibberellic acid made by the firm "Plantprotection" (England) (which we obtained from corresponding member of the Armenian SSSR Academy of Sciences, M. Kh. Chailakhyan, for which we express our thanks) in an experiment with varieties yellow Kishmish, Al'burla, and partially with variety Nimrang.

The experiments were carried out on plantings of the Experimental Production Base of the Magarach VNIIViV near Yalta. It must be noted that the meteorological conditions that year were not especially favorable for grape crops; there was less than normal precipitation during the second half of summer, and a very cool autumn caused the grapes to be retarded in ripening.

Treatments with solutions of gibberellic acid were made three times: first time, prior to flowering by means of submerging the flowers with buds in a solution of the acid for a short time, and the second and third times by spraying the grape clusters at the beginning of growth of the grapes and at the time of their ripening.

The ripened grapes were collected and the following calculations were carried out: the weight of the grapes of each cluster, the number of grapes per cluster, the average weight of the grapes in each cluster, the length and width of the grapes. Average indicators were calculated by experimental variants. The sugar

*The gibberellic acid was sent to us by Doctor V. Shevchik from Prague. We express our thanks to Comrade Shevchik for the attention given us.



The effect of gibberellic acid on the size of grapes of bisexual seedless varieties. a – variety oval Kishmish (left to right): two clusters – control; two clusters treated with a 5 mg/liter solution of gibberellic acid; two clusters treated with a 100 mg/liter solution of gibberellic acid. b – variety round Kishmish (left to right by single clusters): control; treated with 1 mg/liter solution of gibberellic acid; treated with 25 mg/liter solution. c – variety Askeri (left to right by single clusters): control; treated with 5 mg/liter solution of gibberellic acid; 25 mg/liter; 100 mg/liter.

content of the grape sap of each variant was measured by the refractometer method (as a percent of the concentration of dry substance in the sap). The number of clusters by variants ranged from four to ten. Besides this, characteristic clusters for the experimental variants were photographed.

First of all we must note as a general conclusion the very different effect of gibberellic acid on the clusters and grapes of the various varieties of grape.

The following four varieties were taken from the seedless varieties in the test: round, oval, and yellow Kishmish and Askeri. The results of the calculations for these varieties of grape are presented in Table 1 (see figure also).

The average weight of the grapes for oval Kishmish was: control, 1.00 g; for clusters treated with a five mg/liter solution of gibberellic acid, 1.37 g; and when treated with 100 mg/liter solution, 1.98 g; that is, the increase was practically double. For yellow Kishmish, the control was 0.70 g and when treated with a 500 mg/liter solution was 1.18 g. For round Kishmish, the average weight of the grapes in the control was 0.86 g and was 1.67 g when treated with a 100 mg/liter solution.

No increase in the average weight of the grapes was observed for variety Askeri, but the relationship between the length and width of the grape was changed; in the control it was 1.32 and after spraying with a 100 mg/liter

TABLE 1. The Effect of Gibberellic Acid on the Size of Grapes of Bisexual Seedless Varieties

Variety	Concentration of gibberellic acid, mg/liter	Average weight of the grapes, g	Size of grapes, cm		Relation of length to width	Sugar content, %
			length	width		
Askeri	Control	1.45	1.54	1.16	1.32	22.7
	5	1.35	1.55	1.13	1.37	20.1
	25	1.20	1.50	1.02	1.47	21.0
	100	1.42	1.71	1.02	1.66	23.0
Oval Kishmish	Control	1.0	1.33	1.04	1.28	21.0
	5	1.37	1.44	1.12	1.29	26.0
	100	1.98	1.78	1.26	1.41	22.5
Round Kishmish	Control	0.86	—	—	—	18.2
	1	1.15	—	—	—	22.9
	5	1.0	1.12	1.13	0.99	18.8
	25	1.10	—	—	—	18.9
	100	1.67	1.34	1.30	1.03	22.6
Yellow Kishmish	Control	0.70	—	—	—	21.9
	500	1.18	—	—	—	24.4

TABLE 2. The Effect of Gibberellic Acid on the Size of Grapes of Varieties with Functionally-Female Type Flowers (isolated during the entire time of flowering)

Variety	Concentration of gibberellic acid, mg/liter	Average weight of grapes, g	Size of grapes, cm		Relation of length to width	Sugar content, %
			length	width		
Pukhlyakovskii	Control	Grapes did not form and the bush dried out				
	5	1.37	1.47	1.08	1.36	23.3
	25	1.56	1.84	1.24	1.48	23.8
	100	1.84	2.10	1.31	1.60	24.3
	500	2.15	2.11	1.25	1.69	23.1
Nimrang	Control	1.08	1.17	1.18	0.99	24.8
	5	1.41	1.36	1.29	1.05	21.3
	25	1.40	1.47	1.29	1.14	19.8
	100	2.17	1.45	1.21	1.20	17.6
	500	2.61	1.84	1.41	1.30	17.6

solution of gibberellin it was 1.66. Such a tendency was also observed for variety oval Kishmish.

Varieties Al'burla and Royal Vineyard were used for investigation from the bisexual varieties that normally form seeds in the grapes.

For variety Al'burla, the treatment with gibberellic acid caused a decrease in the size of the grapes and practically no change in their form took place. All grapes of this variety, both in the control and in the experimental clusters, had seeds. When bushes of variety Royal Vineyard were treated with solutions of gibberellic acid in concentrations of 5 and 25 mg/liter, there was no increase in the average weight of the grapes, but the number of seedless grapes increased significantly. With this, these grapes had completely normal appearance and dimensions, while the seedless grapes were very small and parthenocarpic for control clusters.

In the experiment with varieties Nimrang and Pukhlyakovskii, which have functionally-female type flowers, part of the treated flowers of these varieties were immediately covered with parchment isolators preventing pollination with foreign pollen. The other part of the flowers remained exposed and were subjected to free pollination.

The results of treatment of varieties Nimrang and Pukhlyakovskii with solutions of gibberellic acid are presented in Table 2.

The treatment with gibberellin solutions led to somewhat of a decrease in the dimensions of the grapes formed under conditions of free pollination, while for variety Pukhlyakovskii, the treatment with a 100 mg/liter solution resulted in somewhat of an increase in the average weight of the grapes.

In the absence of pollination of varieties with functionally-female type flowers under the parchment isolators, where only parthenocarpic grapes could be formed, a very strong positive effect of the gibberellic acid appeared. For Nimrang, the average weight of the grapes in the control clusters was 1.08 g and in the clusters treated with a 500 mg/liter solution of gibberellic acid was 2.61 g. The grapes were, naturally, seedless. For Pukhlyakovskii, isolation of the flowers at the time of flowering resulted in complete drying out of the bush. Treatment with gibberellic acid prior to isolating the flowers results in the abundant formation of seedless grapes, and the size of the grapes increases along with the increase in the concentration of gibberellic acid,

reaching practically a normal size for this variety. The thus-obtained cluster is extremely thick.

The observations carried out obviously showed that treatment with solutions of gibberellic acid also for the varieties forming normal seeds causes significant changes in the formation of the cluster, in the form and size of the grapes, and also sometimes atrophies the seeds, and completely seedless grapes are obtained.

On the basis of the preliminary data that were obtained, it is difficult to reach any specific conclusion about the effect on the accumulation of sugar in grapes which had their flowers and clusters treated with solutions of gibberellic acid. Apparently, the biological features of the different varieties also have a great deal of importance. For example, in Kishmish varieties of grape, a tendency toward an increase in the sugar content of the grapes clearly appears under the effect of treatment with gibberellic acid. The sugar content under the effect of gibberellin is significantly retarded for Nimrang clusters with seedless grape, varieties Al'burla and Royal Vineyard. The sugar content of the grapes of Pukhlyakovskii and Nimrang with free pollination is practically the same in the control and after treatment with solutions of gibberellin, with some exceptions.

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THE EFFECT OF GIBBERELLIN ON THE FRUIT BEARING OF GRAPE, VARIETY CHAUSH

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In recent years an exceptional amount of attention has been paid to the question of the effect of various stimulants on the growth of the higher plants. Foremost among these substances is gibberellin.

Chailakhyan [1] established by investigations that gibberellin affects positively the growth, formation, and generative development of many species of plants, and causes flowering of long-day species under short-day conditions and of biannuals in the first year of planting.

On the basis of their own experiments, Krekule and Martinovska [2], concerned with the effect of gibberellin on the growth and development of winter wheat (variety Codoninska Golitse), summer wheat (variety Niya) and millet (variety Ganatska Mana), came to the conclusion that the effect of gibberellin on the development of plants does not have a universal character. Thus, no effect of gibberellin on the development of short-day millet was observed. Gibberellic acid also did not affect the growth and development of nonvernalized winter wheat. Grebinskii [3] states that gibberellin in small doses increases by two times or more the growth of citrus seedlings, maple, bean, tobacco, tomato, fodder grasses, and others. Along with this, he notes that the growth of the vegetative mass is increased while the yield of grain, tubers, and roots is even decreased.

If the data presented here suggest in most cases a positive effect of gibberellin on the growth and formation of the aerial vegetative organs of many species of plants, then at the present time the question of the effect of gibberellin on fruit bearing remains unclear and experimentally unresolved. Proceeding from this, we established for ourselves the goal of attempting to study the effect of gibberellin on the fruit bearing of grape.

A preparation of gibberellin for the experiments was obtained by us from M. Kh. Chailakhyan, to whom the author expresses his thanks.

We carried out an experiment in 1959 at the vineyard of the Uzhgorod State Grape Farm. Grape variety Chaush was used in the experiments. This is a high-yielding table variety. It is found primarily in mixtures with other varieties in the vineyards of the Carpathians, and only in individual cases in the form of small pure plantings. In contrast to a number of other varieties, Chaush has a functionally-female type flower. These flowers are differentiated from the bisexual by the fact that along with a normally developing pistil, they have short stamens which are bent down with stamen filaments after the fall of the corolla, and have sterile pollen. Consequently, normal fruit bearing for variety Chaush is possible only in the presence of cross-pollination from other varieties.

In our investigation on the effect of gibberellin on the fruit bearing of grape variety Chaush, the experiment was set up as follows: prior to the beginning of flowering, 60 racemes on 20 bushes were selected so that they were as much alike as possible. Parchment bags were placed on all of the selected racemes and they were given numbers.

At the beginning of flowering the test flowers were divided into three variants with 20 flowers in each. Pollen of variety Shasla, which is the best pollinator Chaush, was placed three times (once a day) on the flowers of the 20 racemes of the first variants as the buds opened. In the second variant, the 20 racemes were sprayed during the period of flowering with a 0.002% solution of gibberellin.

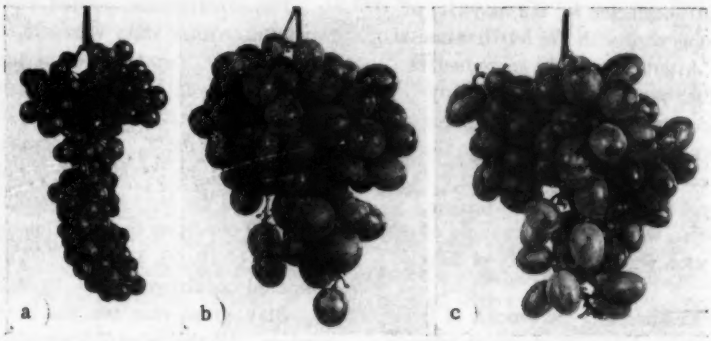
Because flowers on the same raceme did not open simultaneously, the application of the stimulator during a period of about three days resulted in three sprayings on the stigma of practically all flowers of the raceme. Spraying was carried out during the evening when all conditions for preventing the fall of pollen from the air onto the flowers were maintained. Finally, in the third variant, the flowers of 20 racemes were not pollinated and were not sprayed (control).

Fifteen days after flowering, it was already easy to notice the difference in the development of the fruits of the clusters in the experiment. The fruits for variants sprayed with a solution of gibberellin had a lengthened form. The fruit was thicker and longer than for the variant pollinated with pollen from variety Shasla.

The results of the experiments that we carried out were calculated during the harvest period and are presented in the table and in the figure. When the data in the table and the figure are analyzed, we can see that gibberellin when sprayed at a concentration of 0.002% shows a significant effect on the process of fruit formation of grape. All clusters of this experimental variant had normally developed fruits. The average weight of the clusters was only slightly lower than the

Indicators of Development of Grapes in the Individual Experimental Variants

Experimental variant	Average weight of clusters, g	Weight per 100 grapes, g	Volume per 100 grapes, cm^3	Length of the grapes, mm	Width of the grapes, mm	Weight per 100 seeds, g	Sugar, %	Titrated acidity
Pollinated with pollen of variety Shasla	273.0	377	370	20.2	18	3.7	13.5	7.2
Sprayed with gibberellin	268.5	378	364.5	22.8	16.8	No seeds	17.0	5.8
Control	80.7	61.8	51	5.2	5.1	The same	12.0	6.3



The effect of gibberellin on the fruit formation of grape. A) Control – without pollination or spraying (grapes the size of peas); B) pollinated with pollen of variety Shasla (grapes developed normally); C) after spraying flowers with a solution of gibberellin (grapes developed normally).

weight of clusters to which Shasla pollen was applied and significantly exceeded the weight of the control clusters.

The average weight of a single grape in the first two variants of the experiment was similar. The grape in the gibberellin variant had a slightly longer form. The grapes of the variant with spraying did not have seeds and were significantly sweeter to the taste. The increased percent of sugar in the variant with gibberellin spraying apparently is explained by the earlier ripening of the grapes of this experimental variant. One could also judge this because the grapes here were significantly more easily picked from the stems and had a much more expressed gold-yellow coat, characteristic for fully ripened grapes of the Chaush variety.

The results that we obtained give a basis for assuming that gibberellin not only affects the growth of the vegetative organs of many other plants, as has been shown in the literature, but also stimulates the development of the ovule of the flower of grape variety Chaush, which leads to the formation of normally developed, seedless fruit. This also moves up the date of ripening of the grapes.

It would be premature to extend the results which we obtained on the positive effect of gibberellin on the development of fruits of variety Chaush to all varieties of grape. Actually we dealt with an object for which fertilization did not take place and for which there was no such source of growth substances for the ovule as in fertilized seed buds. According to our ideas, the question of the character of the action of gibberellin on the fruit bearing of varieties have functionally bisexual type flowers still needs direct experimental verification.

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THE EFFECT OF GIBBERELLIC ACID ON THE FRUIT FORMATION OF VARIETIES OF GRAPE WITH FUNCTIONALLY-FEMALE TYPE FLOWERS

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The process of fruit formation for the majority of varieties of grape is connected with the fertilization of the seed bud, and any disturbance of the reproductive process, such as the absence of fertile pollen; rainy weather, or low temperatures (below +15°) during the period of flowering, leads to a sharp decrease in the yields. This is observed especially for varieties with a functionally-female type flower (Chaush, Nimrang, Pukhlakovskii and others), which are not capable of self-fertilization in connection with the sterility of their pollen.

A new method for controlling the process of fruit formation is the possibility of the use of growth stimulators. Gibberellin is of particular interest among the growth substances used in horticulture. Because of the detailed study of gibberellin both in our country and in foreign countries, interesting material on its effect on plants has been accumulated. According to the data of Chailakhyan [1, 2, 3], Lang [4, 5] and Phinney [6], gibberellin aids the flowering of long-day species under short-day conditions, changing the photoperiod induction by the long day, and also the flowering of seedlings of winter varieties and biannuals in the first year of life, changing vernalization; dwarf plants under the effect of gibberellin reach the height of normal plants. Weaver [7], treating flowers of grape variety black Korinka with gibberellic acid in concentrations from 5 to 500 mg/liter, observed the formation of grapes that were larger than usual. The work of Wittwer and others [8] and Mosolov and Mosolova [9] established that one can obtain fruits without fertilization (parthenocarpic) when the flowers of tomatoes, peppers, and cucumbers are sprayed with gibberellin in proportions from 10 to 100 : 100,000.

In spite of the fact that evidence of the effect of gibberellin on a whole series of crop plants exists, we still do not have adequate data on its use in viticulture, in particular for obtaining seedless, normally developed grapes – grape varieties with functionally-female flowers. Therefore, experiments were set up by us with the purpose of studying the effect of gibberellic acid on the process of fruit formation of varieties with functionally-female structure flowers, varieties Chaush and Nimrang.

The experimental portion was carried out at the Novo-Dzhankoisk State Vineyard, Crimea district, and at the Salgirk Educational-Experimental Farm of the M. I. Kalinin Crimean Agricultural Institute.

The gibberellic acid used in our experiments was obtained from the K. A. Timiryazev Institute of Plant Physiology of the USSR Academy of Sciences and was used both in the form of water solutions and in the powdered condition in the following concentrations: in solutions of 10, 50, 100, and 150 mg/liter; in the powdered condition of 1, 5, 10, and 15 mg/g.

Clay, grape vine ash, and beet sugar were used as carriers. All the above concentrations were studied both on flowers isolated (with parchment isolators) from pollen of other varieties and on nonisolated flowers (intervariety pollination plus treatment with the growth stimulators). In order to measure the treatment, single and double pollinations or spraying of the flowers being examined were carried out. We made the first treatment of the flowers with gibberellic acid during the period of mass flowering, and the second ten days after the first. Additional variants were included for variety Nimrang, in which the first treatment of the flowers was made five days after the completion of flowering and the second again ten days after the first.

Control variants: 1) free cross-pollination – flowers of variety Chaush pollinated with the pollen of white Shasla Nimrang, with the pollen of a mixture of varieties; 2) flowers isolated from cross-pollination. In each of the variants there were from 10 to 25 test flowers. Solutions of the growth simulator were applied to the flowers with a sprayer and the powdered substances with the help of a hand pollinator. In the spraying, 100 g of the solution was applied to 50 racemes of the Chaush variety and to 30 racemes of the Nimrang variety (the racemes are larger); in dusting, 1 g of the powder treated 25 racemes of the Chaush variety and 15 racemes of the Nimrang variety. The growth stimulator was applied to the flowers during the morning (from 8 to 10 o'clock). In the process of the work the following calculations were made: The number of flowers per raceme was determined, the number of grapes developing per raceme,

TABLE 1. The Effect of the Carriers of Gibberellic Acid on the Process of Fruit Formation of Varieties Chaush and Nimrang

Carriers and solvents	Concentration of gibberellic acid	Percent of flowers forming grapes	Average weight of clusters, g
Variety Chaush			
Distilled water	100 mg/liter	44.0	390
Beet sugar	10 mg/g	48.9	406
Grape vine ash	10 mg/g	49.6	416
Kiel clay	10 mg/g	50.2	381
Control (free cross-pollination)		20.0	170
Variety Nimrang			
Distilled water	100 mg/liter	33.9	655
Beet sugar	10 mg/g	35.0	698
Grape vine ash	10 mg/g	35.8	727
Kiel clay	10 mg/g	30.5	657
Control (free cross-pollination)		17.8	371

Note: In the Table data are presented only on one of the studied concentrations because other concentrations gave the same results of the effect of the carriers.

the average weight of 100 grapes, the average weight per cluster, the average weight of 100 seeds, the number of seeds in 100 grapes, and the percent of sugar, acid and dry substance in the grapes. All of the above calculations were carried out according to standard methods. We present the results below by individual variants of the experiment.

Effect of the Carriers of the Gibberellic Acid on the Percent of Grapes Developing and Average Weight of the Clusters

The results of the effect of the carriers of the gibberellic acid for the double treatment of isolated flowers are presented in Table 1.

Table 1 shows us that the greatest weight of the clusters both for Chaush variety, 416 g, and also for Nimrang variety, 726 g, was obtained on using grape vine ash as a carrier. The absence of sharp variations in the average weight of the clusters in relation to the carriers that were used suggests that, besides grape vine ash, Kiel clay and beet sugar, and water in the case of solutions, can be used successfully as carriers. In connection with this, we present results only for those variants in which grape vine ash was used as a carrier for gibberellic acid(in Tables 2 and 3), in spite of the available data on all of the carriers that were studied.

The Effect of the Concentration of Gibberellic Acid on the Process of Fruit Formation of Chaush and Nimrang Varieties

As the results of the experiments showed, the concentration of gibberellic acid has a large effect on the process of fruit formation. The effectiveness of the action of the growth stimulator with various concentrations is presented in Table 2.

Analyzing the data in Table 2, we come to the conclusion that both the average weight of the clusters and the average weight of 100 grapes are sharply increased with an increase in the concentration from 1 to 15 mg/g. The weight of a cluster was 244 g with a double treatment of isolated flowers with gibberellic acid of a concentration of 1 mg/g, but it was 423 g with a concentration of 15 mg/g; correspondingly, the average weight of 100 grapes increased from 201 to 336 g; at the same time the average weight of a control cluster was 170 g and the average weight of 100 control grapes was 300 g. A structuralanalysis of a cluster showed that the effect of the stimulator on the change in the stems increased significantly with an increase in the concentration to more than 10 mg/g. With increased concentrations, the stems of the grape clusters are lengthened and rapidly become woody, the stems of the grapes lose their elasticity, and this leads to a sharp decrease in the transportability of table varieties of grape. Therefore, one must consider from 5 to 10 mg/g of powdered carrier or from 50 to 100 mg/g of solvent to be the optimum concentrations.

The increased weight of the clusters and the average weight of 100 grapes are obtained with a double treatment of the flowers with gibberellic acid. A noticeable effect of the growth stimulator on the leaves of grape was not observed.

As we indicated earlier, a portion of the variety Nimrang flowers were treated with gibberellic acid (10 mg/g concentration), the first time five days after flowering and the second time ten days after the first treatment. Clusters were obtained in the above variants with parthenocarpic grapes which in form and size differed little from the controls.

TABLE 2. The Effect of the Concentration of Gibberellic Acid on the Fruit Formation of Chaush Variety

Experimental variant	Concentration of gibberellic acid mg/g	Single treatment			Double treatment		
		av. wt. per 100 grapes, g	av. wt. of a cluster, g	% of flowers forming grapes	av. wt. per 100 grapes, g	av. wt. of a cluster, g	filling out of grapes, %
Free cross-pollination plus treatment with gibberellic acid	1	148	155	42.2	261	331	45.5
	5	176	215	45.2	273	336	48.5
	10	191	258	49.8	288	418	59.7
	15	189	251	49.8	304	428	55.9
Flowers isolated plus treatment with gibberellic acid	1	158	182	41.0	201	244	43.0
	5	172	201	43.9	279	344	41.0
	10	180	225	44.5	320	416	49.6
	15	186	236	44.6	336	423	48.9
Control (cross-pollination)	—	297	174	22.1	300	170	20.0

TABLE 3. The Effect of Gibberellic Acid on the Dates of Ripening and Chemical Composition of Grapes of Chaush and Nimrang Varieties (sugar and dry substance in % and acidity in ‰)

Pollination variant	Aug. 13			Aug. 28			Sept. 13			Sept. 28		
	sugar	dry substance	acidity	sugar	dry substance	acidity	sugar	dry substance	acidity	sugar	dry substance	acidity
Variety Chaush												
Racemes isolated + treatment with gibberellic acid	11.9	15.6	3.8	18.9	18.1	3.1	21.4	18.3	2.7	—	—	—
Free pollination + treatment with gibberellic acid	8.6	14.3	5.6	17.3	17.1	4.2	18.7	17.8	3.5	—	—	—
Control (free pollination)	5.2	10.8	12.0	9.6	12.7	10.1	14.5	15.5	6.8	—	—	—
Variety Nimrang												
Racemes isolated + treatment with gibberellic acid	8.1	12.4	12.3	14.8	14.9	6.2	19.8	18.2	3.9	24.1	19 0	3.6
Free pollination + treatment with gibberellic acid	7.0	12.1	13.1	10.3	13.6	8.1	17.1	17.8	5.8	20.4	18.7	5.2
Control (free pollination)	4.2	11.8	22.6	5.3	12.4	20.0	10.6	13.6	18.7	16.6	16.4	12.6

The Effect of Gibberellic Acid on the Percent of Filling Out, Morphological Changes and Chemical Composition of the Grapes

It was established that with a double treatment of the flowers with gibberellic acid in a concentration of 10 mg/g, the percent of grapes developed in the Chaush variety was increased from 20% (control) to 59.7% for the treatment of nonisolated flowers and to 49.6% for isolated flowers. The corresponding percentages for variety Nimrang increased from 17.8% (control) to 38.4% for the treatment of nonisolated flowers and to 33.1% for isolated flowers. It must be noted that on individual racemes the percent of developed grapes increased to 85-90%. This gives a basis to assume that with the help of growth stimulators, one can reach practically 100% development of the grapes in the raceme. All grapes in the cluster were parthenocarpic with treatment of isolated racemes with gibberellic acid; from 15 to 20% of the grapes developed with seeds with treatment of non-isolated racemes, which is connected with the cross-pollination of the racemes prior to treatment with the growth stimulators.

Parthenocarpic grapes obtained when the racemes were treated with gibberellic acid had a long-oval form in comparison to the round form in the control.

Gibberellic acid showed a great effect on the date of ripening of the grapes. Clusters both of variety Chaush and of variety Nimrang whose flowers were treated twice with gibberellic acid ripened from 15 to 20 days earlier than the controls.

Data are presented in Table 3 by variants, the racemes of which were treated twice with gibberellic acid in a concentration of 10 mg/g.

The data that were obtained show that gibberellic acid aids the increase in sugar content and the

decrease in the acidity of the cell sap of the grapes, and also the increase in the percent of dry substance in the grapes. Thus, for example, variety Chaush grapes, the flowers of which were not treated with growth stimulators (control), had a sugar content during the harvest period (Sept. 13) of 14.5%, but the sugar content was 21.4% for grapes from flowers treated with the growth stimulator; the percent of dry substance increased correspondingly from 15.5% (control) to 18.3%. This was also observed for variety Nimrang.

Besides the appearance of an affect of gibberellic acid on the process of fruit formation for varieties with functionally-female type flowers, experiments were carried out to obtain normally developed clusters for strongly dusted clones of variety Riesling. The data that were obtained confirm the possibility of obtaining a normal yield of these clones with a double treatment of the flowers with gibberellic acid in a concentration of 10 mg/g.

In conclusion, we extend out thanks to Professor P. T. Bolgarev for directing the work and to Professor N. Kh. Chailakhyan for the gibberellin for the tests.

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ACCELERATION OF FLOWERING OF SHORT-DAY PLANTS TREATED WITH GIBBERELLIN

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Xanthium, soybean, Kalanchoe, Perilla, and Mammoth*tobacco will not flower on long days when treated with gibberellin [1-5]. Moreover, if Kalanchoe plants growing on short days are sprinkled with a gibberellin solution, the accelerating effect of the short-day treatment is reduced [1a]. On the basis of these results, it was generally assumed that it is impossible to hasten development of short-day plants by treatment with gibberellin. It was suggested that short-day plants contain sufficient natural gibberellins and therefore do not flower earlier upon treatment with gibberellin, as do long-day plants [5]. At the same time, it is well known that both long-day and short-day plants exhibit marked changes in growth and morphogenetic processes as a result of gibberellin treatment. In later studies with Xanthium it was shown that the period of photoperiodic induction could be shortened in this short-day plant by application of gibberellin [6, 7].

It was considered essential to determine whether this were also true with respect to other short-day plants. Experiments were therefore set up with Perilla ocymoides, Italian hemp, and Giant sunflower.

Experiments with Perilla. Plants were grown under continuous illumination for a period of 15 days after sprouting (July 16). They were then divided into two equal groups. One group was sprinkled once with a 0.01% gibberellin solution, and then (July 31) the plants were exposed to different numbers of short days. Control plants, which had not been treated with gibberellin, were exposed to the same regimes. The experimental protocol and the results obtained are presented in Table 1.

It is obvious that the preliminary gibberellin treatment did not induce floral bud formation on a 24-hour day and did not hasten it on a 12-hour day. Such treatment was, however, extremely effective when short days were given for 9-18 days. Control plants exposed to nine short days and then placed on a 24-hour day did not form floral buds. Under the same conditions, plants treated with gibberellin formed buds on the 36th day. In Table 1 it is seen that gibberellin hastened photoinduction in Perilla.

Experiment with Italian hemp. Being a short-day plant, Italian hemp does not tolerate long days during the first 15 days after sprouting [8]. Its photoperiodic reaction is peculiar in yet another way. If it receives a small number of short days which is, however, insufficient to induce long-day flowering, it still shows some response. On such plants, simple leaves appear in place of complex leaves (Fig. 1). It is true that under continuous illumination plants which at first produce simple leaves resume production of complex leaves as growth progresses. Nevertheless, the presence of simple leaves indicates that the plants have been exposed to short days and have responded to them.

The experiment was designed to determine whether treatment with gibberellin would hasten the response of sprouting plants to a short day. From the time of sprouting, plants were exposed to 2, 4, 6, 8, and so on up to 24 short days, after which they were kept on a 24-hour day. One series of plants, the control series, received no gibberellin treatment, while plants of the experimental series were sprinkled with a gibberellin solution daily during the period of short-day treatment.

Each treatment group comprised 15 plants. A month after the short-day exposure period had ended, the number of plants in each group with only complex leaves was counted. These were plants which had not responded to the photoperiodic regime applied. Results are presented in Table 2.

It was shown that control plants reacted almost completely to a short day only when 20-24 such days were given. The same effect was obtained for gibberellin-treated plants at much shorter exposure periods, from 8-12 short days.

It is obvious that the combined action of a short day and gibberellin increases the photoperiodic sensitivity of this plant.

Thus, both the short-day plants Perilla and Italian hemp show a more marked response to short days if treated with gibberellin, either prior to exposure to short days (Perilla) or during the exposure period (Italian hemp).

It might be assumed that in both cases gibberellin acted primarily as a substance stimulating stem growth and axillary bud activity. Both Perilla and Italian hemp, when treated with gibberellin, were taller (about 1.5-2 times). The intense physiological activity of the buds in the axils of the simple leaves of hemp may be judged by the fact that after transfer to a long day they produced vigorous lateral shoots. The same buds on control plants remained dormant.

* Also known as Mamont.

TABLE 1. Number of Short Days to Floral Bud Formation in Perilla Plants Grown under Various Photoperiodic Regimes

Preliminary treatment	24-hour day throughout	No. of short days applied					12-hour day throughout
		6	9	12	15	18	
Control	none	none	none	38	32	26	20
Gibberellin 0.01%	none	none	36	28	24	22	20

TABLE 2. Number of Italian Hemp Plants Which Did Not Respond (did not show an alteration in leaf structure) to the Short-Day Treatment Given

Treatment	No. of short days given from sprouting										
	4	6	8	10	12	14	16	18	20	22	24
Control—no treatment with gibberellin	15	15	15	14	13	11	10	6	3	1	0
Daily treatment with gibberellin during the short-day exposure period	15	13	3	1	0	–	–	–	–	–	–

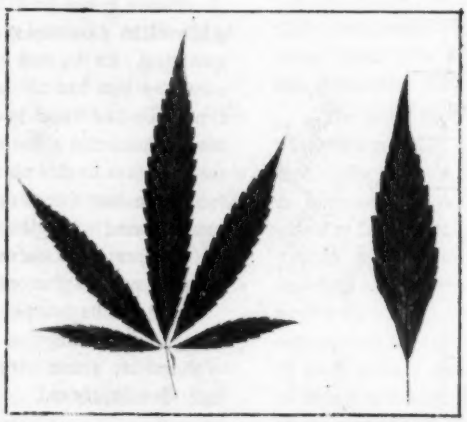

Fig. 1. Leaves from Italian hemp plants which had received eight short days from sprouting. Left – control, right – treated with gibberellin.

Our results are in full agreement with those obtained for Xanthium [6, 7]. The stimulation of bud growth by gibberellin (in Xanthium, Perilla, and hemp) ensures a more rapid response to the photoperiodic regime. Contrary to the opinion of a number of workers, treatment of short-day plants with gibberellin can markedly accelerate their development with short-term exposures to short days.

Experiment with Giant sunflower. This variety of sunflower is a falcultative short-day plant. It flowers earlier on a short day and much later under continuous illumination. Plants were grown on a 24-hour and a 13-hour day. Half the plants in each group were treated daily for 30 days with a 0.01% solution of gibberellin, and the other half was untreated.

Treatment with gibberellin had an extremely strong effect on growth and morphology of the plants (Table 3). Plant height was increased by 20-30% both on a 24-hour day and a 13-hour day. There was a greater number of leaves (8-10) per stem. Leaf morphology was altered markedly. Under the influence of gibberellin the width of the leaf blade was halved. The leaf assumed an elongated oblong-elliptical form. The shape of the base of the leaf at the juncture with the petiole was markedly altered. In place of the normal prominent heart-shaped base was a rounded wedge-shaped structure. Thus, the

TABLE 3. The Reaction of Giant Sunflower to Gibberellin Treatment at Various Day Lengths

Day length	Plant height, cm		No. of days to flowering of 50% of the plants		Leaves						Wet wt. in g					
					No. per plant		width		length		of one leaf		of stems		of heads	
	c*	g**	c	g	c	g	c	g	c	g	c	g	c	g	c	g
24 hr	188	236	No flowering	80	36	46	11	5	15	13	4.0	1.5	194	136	5	46
13 hr	134	189	74	74	30	42	10	5	17	11	4.9	1.3	79	95	83	61

* c – control – no gibberellin applied

** g – gibberellin applied

Fig. 2 Giant sunflower plants grown under continuous illumination. Left – controls; right – treated with gibberellin for 30 days. Treated plants had formed heads.

leaf form characteristic of sunflower was lost as a result of treatment with gibberellin. Under these conditions leaf weight was decreased more than three-fold.

Gibberellin had an extremely pronounced effect on plant development. On the short 13-hour day, which is favorable for development, flowering of both control and treated plants was relatively rapid and occurred in a single burst (on the 74th day).

Plants grown on a 24-hour day and treated with gibberellin flowered on August 23, the 80th day from sprouting. At the end of the experiment, (September 16) control plants had not begun to flower, although at this time they had floral buds (Fig. 2). Thus, under continuous illumination gibberellin hastened flowering by more than 24 days in this plant. It appears from published information that this is the first case in which a short-day plant treated with gibberellin flowered earlier as a result of such treatment under continuous illumination, which is not the optimal photoperiod.

Our results compel a very careful evaluation of the categorical assumption of many workers that treatment of short-day plants with gibberellin cannot accelerate their development.

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PRACTICAL APPLICATIONS

THE EFFECT OF TEMPERATURE ON GERMINATION OF SEED OF KUBAN COMMERCIAL VARIETIES OF RICE

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In the field, rice sprouts usually undergo natural thinning. An investigation of commercial plantings in Kuban has shown that the number of plants varies from 38 to 300 per square meter, with an average of about 150 [1, 2]. The optimal stand density, at which 40-50 centner/hectare can be obtained, is 250-300 plants/m^2 [3]. Therefore, the Kuban varieties fail to produce a significant grain yield because of too low a stand density.

The number of plants per unit area is closely related to seed germination, which depends on temperature. It is therefore necessary to devote special attention to the dependence of seed germination on temperature of the soil and the irrigation water.

It has been established that the greatest number of rice shoots of different varieties is obtained at 22-27° [4]. At the same time, it is currently thought that for sprouting to occur it is necessary to sow at such a time that the average temperature during germination will be 13-16° [5, 6]. This is evidently related to the fact that the temperature minimum at which germination starts ranges from 8-12° [7, 8]; these heat characteristics are the basis of the determination of sowing date. At these temperatures, however, shoots appear sporadically, seedling growth is slow, and plantings thin out [9]. Even so, in practical manuals it is recommended that sowing be completed before the advent of temperatures favorable to germination (11-12) [10], or that it be carried out at a soil temperature of 12-14° [5] or 14-16° [11].

The criterion of correct sowing date is vigor of the sprouts and their density; it is on this basis that a good yield is obtained. Density of plantings depends on the heat regime, however. It has been the purpose of this study to clarify, on the basis of certain physiological indices, the reaction of Kuban commercial varieties of rice to various temperatures in order to establish optimal sowing dates.

Indices of reaction of different varieties to various temperatures which were employed are as follows: water absorption during imbibition, rate of seed germination, growth of shootlets and rootlets, respiration, and cytochrome oxidase activity.

Respiration and cytochrome oxidase activity were determined manometrically [12], and germination rate was determined by a daily count of germinated seeds. The experiments were run in duplicate. Varieties used were: Dubov 129, Krasnodar 424, Krasnoarmei 313, VROS 213, Krasnodar 3352, and VROS 3716.

The chief factors involved in seed germination, other things being equal, are water absorption and respiration. The relation between water uptake and respiration is, however, still unclear [9]. Undoubtedly imbibition is primary in its effect; only after absorption of a certain amount of water does the grain begin to respire actively and thus to regulate physiological processes in the newly active embryo and endosperm. In order to test this assumption an experiment was set up in which 30 seeds of each variety were placed in the central compartment of separate Warburg flasks. A 4.5 ml aliquot of distilled water was added, the total volume occupied by seeds plus water being 4.8 ml. To the center well was added 0.2 ml of distilled water. The amount of water absorbed

TABLE 1. Amount of CO_2 Released (in μ liters per gram dry weight per hour) and Water Absorbed (in % per gram dry weight) by Rice Seeds Germinating at Various Temperatures (variety VROS 3716)

Time (in hr)	9–11°		16–18°		25–27°	
	CO_2	H_2O	CO_2	H_2O	CO_2	H_2O
0**	0.37	12.5	0.37	12.5	0.37	12.5
10	0.37	18.5	0.37	18.5	0.37	18.5
30	22.0	19.0	30.0	21.0	90.0*	23.6*
50	25.0	19.5	52.0	22.0	—	—
70	30.0	20.2	70.0*	23.2*	—	—
90	35.0	20.8	—	—	—	—
110	41.0	21.6	—	—	—	—
130	48.0	22.3	—	—	—	—
150	55.0*	23.0*	—	—	—	—

*Seeds had germinated. **Air-dry seeds.

TABLE 2. Germination of Rice Seeds at Various Temperatures (in %)

Variety	Temperature, °C	No. of days after soaking of seeds							
		2	4	6	8	10	12	14	16
VROS 3716	9—11	0	0	18	60	80	84	90	92
	16—18	0	67	89	—	—	—	—	—
	25—27	95	—	—	—	—	—	—	—
VROS 213	9—11	0	0	25	65	82	83	86	91
	16—18	0	72	86	—	—	—	—	—
	25—27	84	95	—	—	—	—	—	—
Krasnoarmei 313	9—11	0	0	17	60	81	89	93	98
	16—18	0	78	90	—	—	—	—	—
	25—27	87	98	—	—	—	—	—	—
Skorospelyi 8	9—11	0	0	4	45	82	86	91	93
	16—18	0	60	95	—	—	—	—	—
	25—27	67	95	—	—	—	—	—	—
Krasnodar 3352	9—11	0	0	1	2	3	25	80	90
	16—18	0	50	96	—	—	—	—	—
	25—27	55	90	96	—	—	—	—	—
Krasnodar 424	9—11	0	0	1	2	8	22	75	80
	16—18	0	40	87	90	—	—	—	—
	25—27	52	94	96	—	—	—	—	—
Dubov 129	9—11	0	0	1	3	8	20	50	75
	16—18	0	50	87	92	—	—	—	—
	25—27	48	94	—	—	—	—	—	—

by the seeds and the amount of CO_2 released was then determined. The experiment was run in duplicate.

Results indicated that the different varieties begin to germinate after absorption of different amounts of water: VROS 213 begins to germinate at 23% water per gram dry weight, VROS 3716 at 24%, Krasnoarmei 313 at 25%, Dubov 129, and Krasnodar 424 at 28%, and Skorospelyi 8 and Krasnodar 3352 at 30.5-31%. The process of water absorption itself consists of two stages. The first is purely physical in nature and lasts for 7-10 hours. The amount of water absorbed during this time per gram dry weight ranges from 18-20% (VROS 213, VROS 3716, Krasnoarmei 313) to 23-28% (Dubov 129, Krasnoarmei 3352). The amount of CO_2 given off remains at the level characteristic of air-dry seeds. Following this is the second stage, during which imbibition is accompanied by a steady increase in CO_2 released. There is also an increase in amount of water uptake by the grain. This is shown by the data presented in Table 1.

From these data it follows that the first stage is not affected by temperature in the range 9-27°. On the other hand, the second stage is wholly dependent on temperature.

In the first stage, the amount of water absorbed remains unchanged over the range from 9-27°. This water subsequently begins to act as a chemical reagent, catalyzing the activity of the most varied enzyme systems, primarily the respiratory enzymes, which at this time play a basic role in the activity of the embryo, which influences all physiological processes, including water absorption by the seed. Reduced temperatures thus exert an adverse influence on water absorption by depressing respiration.

The low respiratory rate and retarded flow of water into the seed which occur at reduced temperatures in their turn resulted in a very slow germination. This is shown by the data of Table 2.

If varieties are compared with respect to rate of germination at 25-27°, no differences are evident, with the exception of VROS 3716, in which 95% of the seeds had germinated on the second day. At lower temperatures, however, substantial differences between varieties may be observed. These are especially pronounced at 9-11°, at which there is a sharp division of the varieties into two groups. Those of the first group germinate relatively easily at reduced temperatures (VROS 213, VROS 3716, Krasnoarmei 313, and Skorospelyi 8), while those of the second group germinate weakly (Dubov 129, Krasnodar 3352, and Krasnodar 424).

Reduced temperatures also have an adverse effect on growth and other physiological processes of young seedlings. This is related to respiration as a whole and to the respiratory enzymes in particular.

In the initial stages of growth, a large part of seedling respiration is mediated by cytochrome oxidase [1]. An account of its activity in one-day-old seedlings is presented in Table 3.

TABLE 3. Cytochrome Oxidase Activity at Various Temperatures (in μ liters O_2 per gram wet weight after 30 minutes)

Variety	9—11°	16—18°	25—27°
VROS 3716	31	75	128
Krasnoarmei 313	27	69	120
Dubov 129	18	51	127

TABLE 4. Respiration of One-Day-Old Rice Seedlings at Various Temperatures (in μ liters of gas per 60 minutes per gram dry weight)

Variety	9–11°			16–18°			25–27°		
	O_2	CO_2	R.Q.*	O_2	CO_2	R.Q.	O_2	CO_2	R.Q.
VROS 3716	36.0	25.0	0.75	69.0	59.7	0.72	115.0	105.8	0.92
VROS 213	25.4	17.8	0.70	48.3	38.8	0.80	97.0	87.3	0.90
Krasnoarmei 313	29.8	20.9	0.70	44.8	31.4	0.70	89.6	82.4	0.92
Skorospelyi 8	35.0	25.2	0.72	68.2	59.0	0.86	110.0	104.5	0.95
Krasnodar 3352	19.0	14.4	0.60	36.0	21.6	0.60	105.0	86.0	0.82
Krasnodar 424	17.0	10.2	0.60	37.0	24.0	0.65	100.5	75.0	0.75
Dubov 129	16.0	9.3	0.60	32.0	19.2	0.60	100.5	80.2	0.80

*R. Q. – respiratory quotient.

As these data show, reduced temperatures suppress cytochrome oxidase activity, particularly in those varieties which germinate weakly at low temperatures.

As Table 4 shows, respiratory rate is also decreased.

From these data it is seen that respiratory rate and respiratory quotient are highest in all varieties at 25-27°. At 9-10°, however, they are extremely reduced. It is very remarkable that the varieties may also be divided into two groups with respect to respiratory rate, which once more emphasizes the necessity of taking into account their physiological peculiarities.

The small respiratory rate at low temperatures resulted in slow seedling growth. At 25-27°, the length of the roots and the aerial portion on the 16th day was 15 times greater than at 9-11°, and at 16-18° it was ten times greater.

This slower growth was also manifested in the later appearance of successive leaves. Thus, on the 16th day at 9-11° only the coleoptile was present, while at 16-18° the first leaf with a blade had appeared, and at 25-27° there were two or three leaves.

Prolonged exposure of seedlings to reduced temperatures is reflected in a suppression of growth, which may be one of the causes of thinning of commercial plantings.

To examine this possibility an experiment was set up at the Kalinin state farm of the Krasnoarmei district in which Dubov 129 rice seeds were sown on two dates, May 15 and May 30, 1959. Dry seeds were scattered broadcast and the field was then flooded to a depth of 20-25 cm, the water layer being gradually drawn off for better rooting of the germinated seeds. The experiment was evaluated on the basis of the number of shoots with two, three, and five leaves, on special plots one square meter in area. Two plots were sown on each date with an average of seven million seeds per hectare. During the germination period the temperature of the upper 0.5-1.0 cm of soil was recorded.

Temperatures prevailing during the germination periods are as follows (in °C).

Sowing May 15: 17.2; 15.8; 14.2; 14.0; 15.6; 17.0; 17.0; 18.2; 17.5; 16.8; 16.4; 15.8; 16.4; 17.5; 16.7; 17.5; 18.4; 20.2; 21.4· 22.4; 20.5; 21.0;

Sowing May 30: 17.5; 18.4; 20.2; 21.4; 22.4; 20.1; 21.0; 22.0; 21.0; 19.8; 21.0; 23.5.

With the earlier sowing date, shoots first appeared on the seventh day, and the first true leaf appeared only on the 20th day. This is related to the fact that near-optimal temperatures occurred only at the end of May and the beginning of June. With the latter sowing date, however, shoots with the first leaf appeated as early as the 11th day. This difference in the number of days to the appearance of shoots indicates that the most favorable sowing dates should coincide with or should be close to the time at which temperatures optimal for germination occur.

Different temperature conditions were reflected not only in germination rate but in stand density. This is seen from the data of Table 5.

These data show that the stand density was considerably lower with the earlier sowing date than with the later one. Therefore, prolonged exposure of germinating seeds to temperature of the order of 9-16° not only retards growth of roots and aerial organs, but also leads to a significant thinning of the stand.

It may therefore be concluded on the basis of findings with respect to imbibition, germination, and

TABLE 5. Stand Density as Related to Sowing Date (number of plants per m^2)

Stage of shoot development	Sowing date	
	5/15	5/30
Second leaf	82	147
Third leaf	78	142
Fifth leaf	74	136

respiration of seeds of various Kuban commercial varieties of rice exposed to different temperature regimes in the laboratory and in the field at the Kalinin State Farm in the Krasnoarmei district that the optimal sowing date depends on soil temperature and on varietal peculiarities. Such varieties as Krasnoarmei 313, VROS 213, VROS 376, and Skorospelyi 8 are best sown at temperatures in the neighborhood of 16-18°, and Dubov 129, Krasnodar 424, and Krasnodar 3352 at temperatures 2-3° higher.

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RESULTS OF FIELD EXPERIMENTS WITH BARLEY SEED WHICH HAD UNDERGONE A PRE-SOWING HARDENING TO DROUGHT

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The studies of P. A. Genkel' and his co-workers have established that the method of pre-sowing hardening of plants to drought which was proposed by him induces profound physiological changes which result in an increase in drought resistance and in productivity. The theoretical bases and practical features of pre-sowing hardening of individual crops, and also the results of physiological studies, have been given in detail in the papers of Genkel' and other workers [1-7]. A test of the hardening method under field conditions using spring wheat [8, 9] yielded positive results.

The purpose of this study was to examine the effect of hardening to drought on growth, development, and yield of barley under field conditions. Experiments were set up on the state farm "Roshcha" in the Michurinsk district of the Tambov region in 1957-1959.

In Tambov there is often an unequal distribution of precipitation during the growing period, and as a result plants suffer from drought to a greater or less degree.

Two varieties of barley, Nutans 187 and Kazan 6/4, were studied. The average water content during hardening was 50% of the air-dry weight of the seed. Drying of germinated seed was carried out in the air at temperatures from 14-22°. Seed hardening (single treatment) took from four to seven days depending on weather conditions. The scheme of the experiments was as follows:

1) Control plants (without treatment); 2) hardened plants. In 1959 a third treatment was introduced, hardening with boron. Concentration of boric acid was 110 mg per liter of water. Seeds were sown with a tractor seed plow in rows. Experimental plots varied in size from 8 to 15 hectares. Cultivation techniques employed were those of the state farm.

Throughout the growing period gross observations were made relating to growth, development, and yield of the plants. Results of these observations are presented in Table 1.

The growing period in 1957 and 1959 was characterized by considerable drought. In the first two thirds of May , 1957, total precipitation was only 20.3 mm. The minimum air moisture content was 17-20%, and the maximum temperature was 28-30.5°. Thus, a condition of drought prevailed in May, 1959; this continued until the middle of June. In 1959, July was extremely dry, the total precipitation being 18.9 mm; minimum air moisture content was 18-20%, and maximum temperature was 33-34°.

As Table 1 shows, shoots of hardened plants appeared a day or two earlier than those of control plants. The hardening method hastened the formation of leaves in the beginning stages of development, which in turn exerted a beneficial effect on grain production under drought conditions. The growth of experimental plants was also more rapid, as shown by the 1959 data.

1959 Expt.

Exptl. treatment	Plant height, cm (average of 100 plants)
On May 4	
Controls	5
Hardened	7.6
Hardened with boron	7.8
On May 26	
Controls	25.8
Hardened	27
Hardened with boron	26.9
On June 25	
Controls	53
Hardened	54.8
Hardened with boron	55

The superior growth of the hardened plants was especially pronounced at the beginning of the development period and was easily visible to the eye.

At the end of the growing period, the grain yield was determined. The hopper weight of grain from the combine was calculated on the basis of plot area. The data of Table 2 show that yield of the hardened plants was greater than that of the control plants. The pre-sowing hardening treatment had the greatest effect in 1957, when drought conditions were fairly severe in May, at the beginning of plant development. In 1959, hardened plants produced 4 centner/hectare more grain than control plants. An additional increase of 1 centner/hectare was obtained if seeds were hardened with H_3BO_3, which fully confirmed the results of Shkol'nik [10].

TABLE 1. Gross Observations of Barley Development

Treatment	Sowing date	Shoots appear	Third leaf	Tillering	Shoot elongation	Heading	Flowering	Milk stage	Waxy stage	Date of harvest
				1957						
Controls	29.IV	5.V	16.V	22.V	28.V	4.VI	10.VI	29.VI	20.VII	30.VII
Hardened	29.IV	7.V	15.V	22.V	28.V	3.VI	9.VI	29.VI	20.VII	30.VII
				1958						
Controls	21.V	26.V	31.V	6.VI	13.VI	21.VI	26.VI	14.VII	4.VIII	12.VIII
Hardened	21.V	25.V	30.V	6.VI	13.VI	20.VI	25.VI	14.VII	4.VIII	12.VIII
				1959						
Controls	23.IV	2.V	12.V	18.V	26.V	2.VI	7.VI	25.VI	16.VII	24.VII
Hardened	23.IV	30.IV	11.V	18.V	26.V	1.VI	6.VI	25.VI	16.VII	23.VII
Hardened with boron	23.IV	30.IV	11.V	18.V	26.V	1.VI	6.VI	25.VI	16.VII	24.VII

TABLE 2. Yield Data

Treatment	Area, hectares	Grain yield over the whole area, kg	Yield in centner/hectare	%
1957				
Controls	4	7200	18	100
Hardened	4	1200	28	155.5
1958				
Controls	15	24210	16.14	100
Hardened	10	21320	21.32	132.1
1959				
Expt. I				
Controls	2	5000	25	100
Hardened	10	29000	29	116
Hardened with boron	1.5	45 00	30	120
Expt. II				
Controls	2.8	6720	24	100
Hardened	12.2	34160	28	116.6

SUMMARY

A pre-sowing treatment of seed by Genkel's method exerts a beneficial effect on barley growth, development, and grain yield. Such treatment is most effective when there are spring and summer droughts after appearance of shoots. The effectiveness of the method is enhanced if the seeds are hardened by soaking in a boric acid solution. The method can be recommended for extensive trials under field conditions

I wish to thank V. Ya. Timoshchenko, director of the state farm "Roshcha", and V. K. Zhurikhin, agronomist, for their participation in this study of the application of the method of hardening barley to drought to commercial practice.

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A CHEMICAL MICROMETHOD OF ETHYLENE DETERMINATION

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It has been established that the hydrocarbon ethylene is an important factor in the normal economy of fruits [1, 2, 3] and is a highly active promoter of fruit maturation [1-5]. Moreover, it is produced in relatively large amounts by the flowers of a number of plants [6, 7] and may be found as a metabolic product of other parts of the plant [6, 8, 9]. All this explains why the problem of determining microquantities of ethylene has repeatedly attracted the attention of scientists.

For the determination of ethylene produced by plant tissues and also for measurement of this compound in gaseous mixtures, Rakitin [1, 3] has proposed a highly sensitive biological method based on the ability of ethylene to alter the growth rate of bean seedlings and to elicit the epinastic response in leaves of tomato, potato, and other plants.

Chemical methods of ethylene determination have also been recommended. One such method was developed by Christensen et al. [10]. With this method, the preliminary separation of ethylene from other volatile materials was accomplished by a severe cooling. Young et al. [11], Uota [12], Waggoner and Diamond [13], and Phan Chon-Tôn [14] recommend that this be carried out by absorption of ethylene in mercuric chloride.

Having resolved to develop a feasible chemical micromethod of ethylene determination which would be sufficiently accurate, we adopted the method of trapping ethylene in a solution of meruric nitrate in nitric acid and then freeing it with concentrated hydrochloric acid, as originally proposed by Treadwell and Tauber [15] and later adapted to their studies by Hansen and Hartman [16] and Denny [17]. We selected the solution of mercuric nitrate in nitric acid since it is a more convenient reagent to use than mercuric chloride. For the quantitative determination of purified ethylene, we at first tried absorption with a solution of $KBrO_3$ and KBr followed by titration with hyposulfite, as proposed by Christensen et al. [10].

Upon comparing various methods of absorption of purified ethylene, we selected one using permanganate, as employed in the experiments of Nilson [18]. An examination of this method convinced us that it is sufficiently sensitive and that it gives repeatable results with successive titrations. Our method of determining ethylene is described in detail below. Fruits of various plants served as experimental material for ethylene determination.

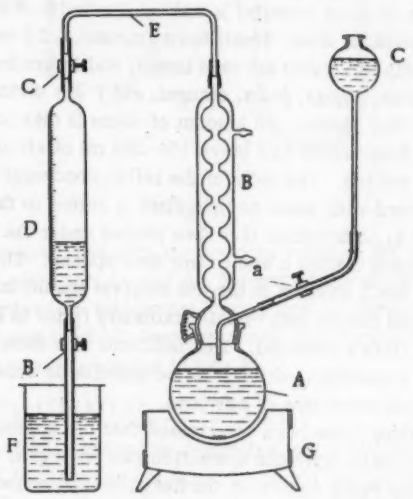

Fig. 1

The method consists of three stages: 1) extraction of ethylene from the fruits; 2) purification of ethylene by absorption in mercuric nitrate followed by release of the gas with concentrated hydrochloric acid; 3) oxidation of the ethylene with potassium permanganate and determination by back titration of an excess of Mohr's Salt.

1. Methods of Extracting Ethylene from Plant Materials

There are two ways of extracting ethylene from plant materials: 1) by boiling [1], and 2) by the use of a mercury pump [19].

a) Extraction of ethylene by boiling. This is a convenient method which utilizes a specially designed apparatus [1] consisting of the following parts (Fig. 1): a 1000-1500 ml round-bottomed distillation flask A; a reflux condenser B with a ground glass joint for union with flask A at the lower end and a short rubber tube at the upper end; a 300-ml glass thistle tube C connected to the lateral glass tube from flask A by a rubber tube fitted with a screw clamp a; a 300- to 400-ml graduated gas receiver D fitted with rubber tubing at each end and screw clamps b and c; a glass tube E connecting the reflux condenser B and the gas receiver D; a 1000-ml beaker F; an electric hot-plate G.

The beaker F is filled one-third full with a saturated solution of NaCl, and the thistle tube C is filled with

water; during this operation the rubber tube from the thistle tube should be closed off with screw clamp a. The gas receiver D is filled with a saturated solution of NaCl; in order to prevent the solution from flowing out, screw clamps b and c are closed at this time. The gas receiver is then fastened to a vertical support. The lower rubber tube from the gas receiver should be immersed in the NaCl solution in the beaker F. Three hundred ml of water are poured into flask A and then a sample of plant material is added. For fruits, 300-400 g of material is used. Small fruits (currant, wild rose, mountain ash fruits) are used intact, and larger fruits (tomatoes, apples, pears, oranges, etc.) are divided into two to four pieces. An amount of water is then added to the flask which will leave 150-200 ml of air above the liquid surface. The joint of the reflux condenser is moistened with water and the flask A united to the condenser B. A hot-plate G is then placed under the flask.

Screw clamps b and c are then opened. The level of the NaCl solution in the gas receiver should fall somewhat and should then remain stationary (prior to heating of the flask's contents). This indicates that there are no leaks, a condition which must be satisfied in order to carry out extraction of ethylene.

When it has been ascertained that the apparatus is free of leaks, ethylene extraction can be begun, the first step being to turn on the hot plate and connect the condenser to the water supply. As the flask's contents are heated and the gases escaping from the plant sample sweep out the air from the flask and the condenser, the salt solution in the gas receiver flows out into the beaker F. The contents of the flask are boiled for 50 minutes, counting from the time that boiling begins.

Ten minutes after boiling begins, screw clamp c should be closed and the hot-plate should be disconnected and removed from under the flask for 3-5 minutes. During this time there is a cooling of the system and a partial vacuum is created in the flask and the condenser, as indicated by the fact that accumulated condensate is drawn from the condenser into the flask. The creation of a vacuum accelerates the evacuation of gases from the fruit tissues. Screw clamp c is now opened and the hot plate is once more placed in position and turned on. After a further ten minutes this operation is repeated, and again after another ten minutes.

At the end of the boiling period (50 minutes), the hot-plate is disconnected, screw clamp a is opened, and all gas is forced out of the flask and condenser into the gas receiver by water flowing from the thistle tube C, which is elevated to facilitate the operation. Screw clamps b and c must be closed immediately after this and the gas receiver disconnected from the apparatus. It is necessary for calculating the volume of free space above the liquid in the flask and the volume of the gas receiver that at the end (after boiling and sweeping of gases into the receiver) not less than 50 ml of NaCl remains in the gas receiver.

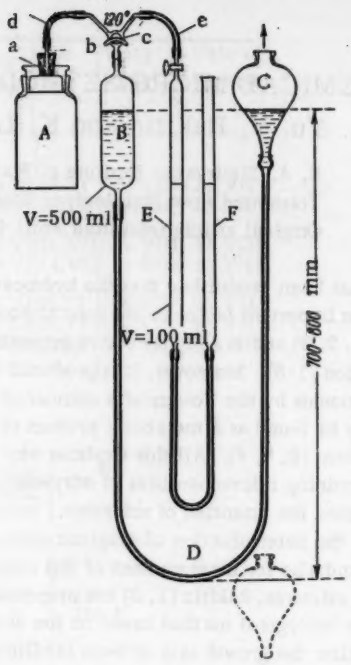

Fig. 2

The weight of the sample analyzed and the volume of the gaseous mixture in the receiver should be accurately measured, since they are used in subsequent calculation.

b) Extraction of ethylene with a mercury pump. For extraction by this method, it is recommended to use a mercury pump constructed by one of us [19], which can be easily duplicated in any laboratory.

This pump (Fig. 2) consists of the following parts: a 500-1000-ml evacuation chamber A with a ground glass stopper a through which there passes a rubber stopper with a glass outlet tube b; a 400-500-ml gas receiver B supplied with a stopcock c with two outlets to which are attached thick-walled rubber tubes d and e; an 800-1000-ml obconical glass container C; a thick-walled rubber tube D about 2 meters long joining B and C; a 100-150-ml gas measuring burette E. All parts of the pump are mounted on a wooden or metallic stand.

With chamber A disconnected, about 1000 ml of mercury is poured into container C. In order to suppress evaporation of mercury, about 100 ml of distilled water is then poured into C. The throat of C is closed with a cotton plug to prevent entrance of dust. Container C is then raised to fill the gas receiver (to the stopcock) with mercury. The rubber tube d is then immersed in a beaker containing a saturated NaCl solution and about 150 ml of this solution is drawn into the gas receiver by lowering container C. Without removing tube d from the salt solution, stopcock c is then closed. Container C is then raised and stopcock c is

alternately turned so as to connect the gas receiver B with tubes d and e; in this way the tubes are filled with the salt solution, after which the stopcock is closed. Saturated NaCl solution is poured into burette E through the leveling tube F. During this operation the burette should be disconnected from the apparatus and its stopcock should be open. An amount of salt solution should be poured in to fill E and F somewhat more than half full. Burette E is filled completely by raising F and its stopcock is then closed. The burette is joined to tube e. Chamber A is filled about $^1/_2$ or $^2/_3$ full and the sample to be analyzed, weighing 250-500 g, is then added. If the sample consists of fruits which will pass through the opening, the fruits are left intact. The ground glass surface of the bottle neck and its stopper are smeared with vaseline. The chamber is then filled to the top with salt solution and the stopper inserted. During this operation the salt solution fills the outlet tube and frequently overflows. The rubber tube d is then connected to tube b. Container C is lowered to the position shown by dotted lines. This creates a vacuum in the gas receiver B. Stopcock c is opened to connect the gas receiver with chamber A. A vigorous evolution of gases from the plant material begins at once, the gases being swept into the gas receiver B.

TABLE 1. A Comparison of Different Methods of Extracting Ethylene from Plant Material

Plant material	Content of ethylene (in ml) per kg fruit	
	boiling	mercury pump
Paper rennet apples	0.52	0.54
Washington Navel oranges (with the rind)	0.66	0.61

Only when gas evolution has subsided markedly, which usually occurs after 1-2 minutes, is stopcock c turned and the stopcock of the burette opened to connect it with the gas receiver; container C is then raised and the gases swept from the gas receiver into the burette. Stopcock c is then turned to close off the burette from the gas receiver, and container B is returned to its lower position, a vacuum thereby being created in the gas receiver. Stopcock c is once more opened so as to connect chamber A with the gas receiver, and evolution of gas from the fruit sample is resumed, the gas being collected in the gas receiver, from which it is then swept into the burette. All operations are once more repeated in the same order. The pumping of gas is continued until its volume in the burette shows little substantial change with each successive gaseous discharge. Usually not more than five to seven repetitions of the total operation

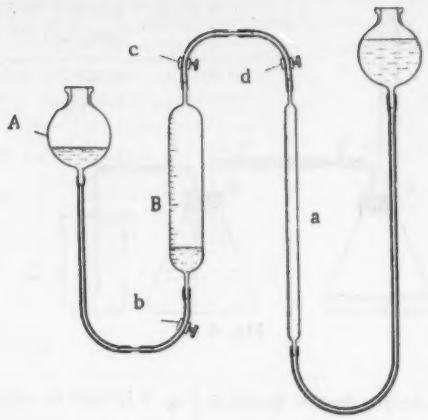

Fig. 3

are required. As with the previous method, the weight of the plant sample and the volume of gas obtained are determined for subsequent calculation. A comparison of the two methods described shows that results obtained are in good agreement (Table 1).

2. Purification of Ethylene

Before quantitative determination of ethylene contained in the gases collected by one of the previously described methods, a sample of gas should be freed of other volatile materials which are unavoidably obtained during extraction of ethylene from plant tissues.

Two reagents are necessary for the purification procedure: a solution of mercuric nitrate, $Hg(NO_3)_2$, in nitric acid, and concentrated HCl. The first reagent is prepared by dissolving 20 g $Hg(NO_3)_2$ in 2N HNO_3. This reagent must be stored in a dark container with a ground glass stopper. It is recommended that it be used not more than 10-12 days.

A sample of gas for purification is first taken. If the ethylene was extracted by boiling and the gaseous mixture is confined in a gas receiver B, the sample is removed using apparatus illustrated in Fig. 3. The gas receiver is connected to a 100-ml burette a and, at its lower end, to a leveling flask A. Before the gas sample is collected, the burette, leveling flask, and connecting rubber tubes should be filled with a salt solution. When this is done, it is necessary to adjust the gaseous mixture in the gas receiver to atmospheric pressure. Screw clamp b should be opened and the liquid levels in flask A and the gas receiver set equal by altering the position of A; when this is done, the volume of gas in the gas receiver should be recorded. Subsequently screw clamps c and d are opened and a sample of gas, usually 100 ml, is forced into the burette by adjustment of the positions of the two leveling flasks. Where ethylene was obtained with a mercury pump and the total volume of gas collected is several times smaller, a smaller sample is taken.

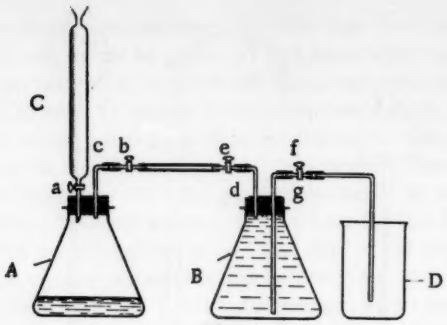

Fig. 4

The simple device shown in Fig. 4 is used in ethylene purification. The component parts are: two 250-300-ml thick-walled flasks A and B which are of exactly the same volume; rubber stoppers in the necks of these flasks; a graduated funnel C with a stopcock a; a 300-350-ml beaker D; a stopcock b and glass and rubber tubing. Through the stopper of flask A there pass the outlet tube of the funnel and a glass tube c. To the latter is attached a length of vacuum tubing and in turn a glass tube with a stopcock b. Through the stopper of flask B there pass a glass tube d joined to a length of vacuum tubing fitted with a stopcock e and a glass tube g with a stopcock f.

One hundred ml of mercury reagent is introduced into flask A and the stopper is inserted. With stopcock a closed, air is evacuated from the flask so as to create a pressure of about half an atmosphere. The partial vacuum created ensures the withdrawal from the gas receiver into the flask of a 100-ml gas sample. If, however, the sample of gas taken is smaller, then a smaller vacuum is required. The flask is then connected to the burette through stopcock b and the gas sample is withdrawn. Stopcock b is closed and the flask is vigorously shaken for 15 minutes in order to promote absorption of ethylene by the mercury reagent. Stopcocks a and b are then opened, the flask is connected to an aspirator pump through the outlet tube with stopcock b, and air is circulated through the flask for 5 minutes. This ensures removal of gases extracted from the plant material which are not absorbed by the reagent.

Stopcock a is now closed, a vacuum is again created in the flask, and 40 ml of concentrated HCl is poured into the graduated funnel. Stopcock a is then opened and the acid is introduced into the flask, after which the stopcock is immediately closed. The flask is shaken vigorously on a shaker for 5 minutes for removal of the ethylene from the mercury reagent. Stopcock b is then quickly opened and closed in order to eliminate the residual vacuum. Flask A is connected with flask B, which is filled with KOH through the tubing with stopcocks b and e, and the gas is forced from flask A into flask B by introduction of distilled water through the funnel into flask A. The solution forced out of flask B runs into the beaker D. Flask B is now disconnected from flask A at stopcock e and is shaken several times to ensure complete absorption of HCl vapors by alkali. Tube g with stopcock f is connected by a rubber tube to a glass thistle tube filled with distilled water, and the free end of the glass tube with stopcock e is connected to a burette filled with a saturated solution of NaCl.

The ethylene gas freed of extraneous contaminants and HCl vapors is then forced into the burette in 20-30-ml portions, depending on the size of sample desired for analysis, from which it is passed into a flask containing $KMnO_4$. It is necessary to know for future calculation the final volume of gas in the flask. With a mercury reagent volume of 100 ml and a flask B volume of 250 ml, it is usually possible to take four or five parallel samples of 20 ml.

3. Determination of Purified Ethylene

The following reagents are necessary for determination of purified ethylene: 0.02 N $KMnO_4$, 1N H_2SO_4, 0.04 N solution of Mohr's salt. Fifty-ml conical flasks fitted with ground glass stoppers with glass stopcocks are used (Fig. 5). It is necessary to smear the stopcock with vacuum grease, to moisten the inner stopper surface with water, and to smear the juncture of stopper and flask with a layer of plasteline.

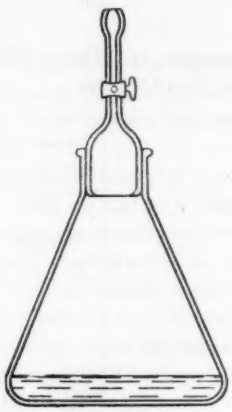
Fig. 5

Into each stopper 5 ml of 0.02 N $KMnO_4$ and 1 ml 1N H_2SO_4 are introduced from a microburette. A vacuum is then created in the flask and a 20-ml sample of gas is introduced. During introduction of the gas sample the stopcock is open. For each analysis it is recommended that three parallel samples be taken.

All flasks are then placed on a shaker and shaken vigorously for 50 minutes. If they are shaken for shorter times, oxidation of the ethylene by permanganate may be incomplete (Table 2). During introduction of the gas sample from the burette, care should be taken that no NaCl solution is allowed to escape, since there is an evolution of chlorine gas under these conditions which vitiates the results of the analysis.

TABLE 2. Determination of Ethylene with Various Shaking Periods at Room Temperature (0.0740 ml ethylene taken for analysis)

Shaking time	Ethylene determined, ml	% of total recovered	Shaking time	Ethylene determined, ml	% of total recovered
5	0.0160 0.0168	22.7	30	0.0521 0.0460	66.2
10	0.0208 0.0214	28.6	40	0.0668 0.0672	90.5
20	0.0320 0.0300	41.8	60	0.0730 0.0727	98.5

TABLE 3. Data from Parallel Titrations with $KMnO_4$

Material	ml 0.02 N $KMnO_4$	Material	ml 0.02 N $KMnO_4$	Material	ml 0.02 N $KMnO_4$
	5.14		5.38		622
	5.20	Gaseous mix-	5.40	Mixture of	6.26
Control deter-	5.16	ture from	5.46	ethylene	6.26
mination	5.16	the Common	5.38	and air	6.27
	5.18	Antonovka	5.42		6.21
	5.14	apple	5.40		6.24
Average	5.16± 0.017		5.40± 0.020		6.24 ± 0.018

At the end of the shaking period, the stopcock is opened, the stopper is removed and its inner surface washed with water, which is allowed to run into the flask; the plasteline is carefully wiped off, 4 ml of a solution of Mohr's salt (an excess) is introduced from a microburette, the flask's contents are heated on an electric hot-plate, with care being taken to prevent boiling, and the excess of Mohr's salt is titrated with a 0.02 N solution of $KMnO_4$.

In view of the fact that in the air of the laboratory, especially in large cities, there may be small amounts of ethylene, we recommend that a control determination be made, all manipulations from absorption by the mercury reagent to titration with $KMnO_4$ being repeated. Average values for control titrations are subtracted from average values for experimental determinations.

As indicated above, parallel titrations with $KMnO_4$ yield results in good agreement with each other (Table 3).

The calculation of ethylene content of plant material is made according to the following formula:

$$\frac{(A-B)\cdot K\cdot V\cdot V_2\cdot 1000}{P\cdot V_1 V_3},$$

where A – ml permanganate used in titration of the experimental sample;

B – ml permanganate used in titration of the control sample;

K – coefficient for conversion of ml permanganate into ml ethylene; 1 ml 0.02N $KMnO_4$ corresponds to 0.0265 ml ethylene;

P – weight of plant material analyzed (in grams);

V – original volume of gaseous mixture (in ml);

V_1 – volume of gaseous mixture taken for absorption of ethylene by the mercury reagent (in ml);

V_2 – volume of gaseous mixture after release of ethylene by HCl (in ml);

V_3 – volume of gaseous mixture taken for oxidation by permanganate;

1000 – factor for conversion to 1 kg of fruit.

Let us make a sample calculation: 250 g (P) of apples yielded 380 ml (V), of gas; 100 ml of gas were analyzed (V_1); gas volume after purification with mercury reagent, release with HCl, and removal of HCl vapors with alkali was 130 ml (V_2); 20 ml of gas were taken for determination (V_3). Titration of experimental samples (A) required an average of 5.44 ml, and titration of control samples (B) an average of 5.16 ml. The coefficient for conversion (K) of the $KMnO_4$ solution titer is 0.0244; the content of ethylene in ml per kg of apples is therefore equal to:

$$\frac{(5.44-5.16)\cdot 0.0244\cdot 380\cdot 130\cdot 1000}{250\cdot 100\cdot 20}=0.649 \text{ ml}$$

per kg of fruit.

This method of determination is good to 0.004 ml of ethylene in a sample. In Table 4 are presented results of determinations of ethylene in mixtures with air and in combinations of air-ethylene mixtures with gaseous samples from fruits.

TABLE 4. Recovery of Ethylene Added to Gaseous Mixtures from Fruits

Material determined	Taken, ml	Found, ml	Percent recovery	Material determined	Taken, ml	Found, ml	Percent recovery
Mixture of ethylene with air (in ml)							
5	0.0150	0.0139	92.6	Gaseous mixture from			
5	0.0150	0.0142	94.6	Common Antonovka	—	0.0284	—
10	0.0300	0.0269	89.7	apple (10 ml)			
				Mixture of ethylene	0.0600	0.0613	102.1
15	0.0450	0.0416	92.4	with air (20 ml)			
15	0.0450	0.0404	89.8	Gaseous mixture from the same apples (10 ml) + mixture of ethylene with air,			
20	0.0600	0.0562	93.7	total 30 ml	0.0884	0.0897	101.3
20	0.0600	0.0611	101.8	Gaseous mixture from			
				rinds of Gruzin	—	0.0235	—
				lemons (10 ml)			
				Mixture of ethylene	0.0250	0.0246	98.6
				with air (10 ml)			
				Gaseous mixture from rinds of Gruzin			
				lemons (10 ml) +	0.0485	0.0471	97.2
				mixture of ethylene			
				with air (10 ml), total			
				20 ml			

TABLE 5. Ethylene Content of Fruits (in ml per kg)

Material	Ethylene content	Material	Ethylene content
Common Antonovka apples — end of August	0.649	Wild rose (R. Motshchin) — rose colored	0.854
The same — after three months' storage	0.390	The same — mature	0.245
Scarlet anise — end of August	0.517	Erliana Gribov tomatoes — rose-colored	0.042
Shtreifling anise — the same	0.412	The same — more mature	0.026
Chinese anise — the same	0.310	The same — mature	0.014
Liya black currant — dark brown, fertile	0.373	Unshiu mandarins — flesh	0.057
The same — ripe	0.245	The same — rind	0.383
		Washington Navel oranges with the rind	0.066

In Table 5 are presented results of our analyses of various fruits for ethylene content.

Our results for apples and other fruits are in good agreement with published data [1, 10, 13].

SUMMARY

The micromethod proposed may be used for determination of ethylene in fruits and other plant tissues in the air of ethylene chambers, in fruit and vegetable storage vaults, in laboratories and commercial establishments, and in samples of air from open places (forests, fields, streets, industrial areas, etc.).

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A DEVICE FOR MICROSCOPIC OBSERVATIONS DURING COOLING AND FREEZING

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Microscopic observations of cooled material have been made by a number of workers using various cooling devices. The simplest method is that of cooling the microscope, which is enclosed in a special box, with an ice-salt mixture, as done by Molisch [1] and other workers [2, 3]. Some workers have used dry ice instead of the ice-salt mixture [3, 4, 5]. A more refined method consists of cooling with a stream of cold liquids or gases; for this purpose liquid carbon dioxide [6], nitrogen [7], or propane [8] have been used, or liquids of low freezing point (solutions), these being cooled by various methods [4, 8-13]. Mixtures of acetone or alcohol with dry ice [8, 10] or ice-salt mixtures [4, 12, 13] have been employed.

Some workers [14-17] have effected cooling with liquid nitrogen or liquid air, not by circulation through a cooling device, but by the use of metals of high heat conductivity. Finally, some workers have used the microscope in a cold room [18-20], the temperature of the object studied being regulated by changing the temperature of the room or by enclosing it in a supplementary electrically warmed device. The form of chamber used has been dependent on the method of cooling.

For microscopic observations of freezing plant tissues, it is extremely important to be able to regulate cooling rate, maintain low temperatures accurately, and suppress or promote supercooling as desired, since the character of ice formation in tissues and its effect on cells depends on these factors. Since the majority of cooling devices fail to meet these requirements, we were obliged to construct a special cooling device (see Fig. 1).

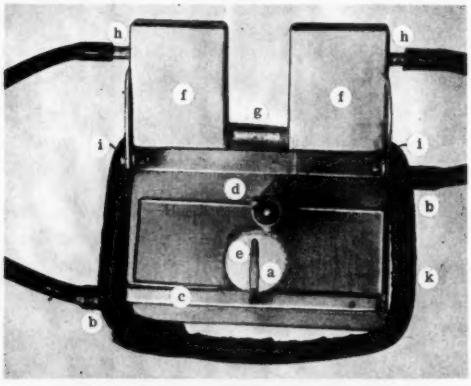

Fig. 1. Cooling attachment

This device consists of a hollow soldered copper box 80 × 55 × 10 mm with an aperature a 20 mm in diameter in the center for passage of light and with two tubes b soldered to it for entrance and exit of the cooling liquid. To the upper surface is soldered a copper strip c 5 mm wide which is of the thickness of a microscope slide and a spring clamp d which holds a thermocouple in contact with the slide for control of temperature of the slide surface in the neighborhood of the object studied. The slide with the object e is placed firmly over the metal strip c, and the object studied is placed so as to be over the aperture a.

Another part of the device functions to prevent clouding over of the cover glass and also to promote cooling of the upper surface of the slide. It consists of two hollow copper boxes f 40 × 30 × 8 mm connected by tube g, each having another tube h in addition. These boxes are attached by rods through small lateral projections to bars i from the main box; they may thus be retracted upward as shown in Fig. 1, when it is necessary to remove the slide, or they may be lowered and appressed to the slide when it is necessary to begin cooling. The sides of the large box are furnished with heat insulation k. The whole device in assembled form is placed on the microscope stage.

Cooling is effected by circulation of a cold liquid of low freezing point, such as 80% methyl alcohol, which is used here; salt solutions of corresponding concentrations and antifreeze, or ethylene glycol, are also suitable.

For cooling of the circulating liquid a refrigerating unit is used consisting of a compressor (for this purpose an FAK-07 compressor is suitable) and a 50-liter metal bath filled with the cooling liquid in which a cooling coil from the compressor is immersed. The unit is plugged into the line through a contact thermometer which automatically maintains a low temperature in the liquid. A small circulation pump from an ultrathermostat, which is run by a motor outside, is also included in the bath. The bath should be covered and should be well insulated on all sides. The refrigerating unit and the microscope are at ordinary room temperature. An overall view of the whole unit is shown in Fig. 2.

The cooled liquid is forced by a pump through insulated tubes, then through a Y-tube into the freezing attachment, where it is divided into two streams, one of which passes through the main box and the other through the two smaller boxes; the two streams are then reunited

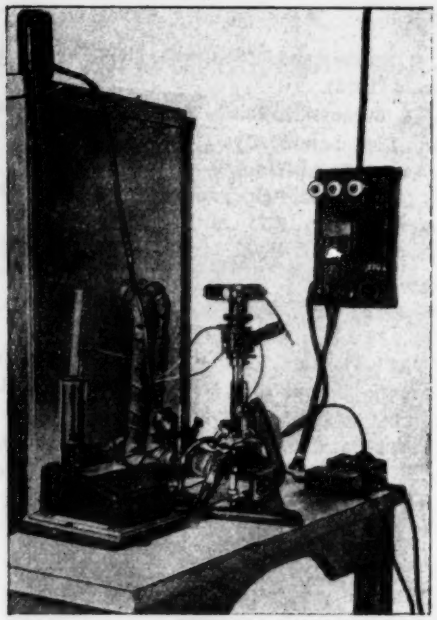

Fig. 2. View of the assembled device for studying plant tissues during cooling and freezing.

through a second Y-tube and the liquid flows into a hollow metal cylinder screwed into one of the openings of the objective holder, from which it is returned through tubing to the refrigerating bath. The objective holder and the objective are thus subjected to cooling. This is of great value, since if the objective is warm it heats up the cooled object (especially if the working distance of the objective is small), and this may vitiate results of determination of the object's temperature by the neighboring thermocouple. The object studied also suffers heating effects from the illumination source; to prevent this, a heat-absorbing filter is interposed between the light and the mirror.

After the slide has been placed in position, the small cooling boxes have been appressed to it, and the objective has been lowered between them and focussed, it is necessary to cover the remaining spaces with cotton in order to prevent a possible fogging over of the cover glass.

The rate of cooling of the object and its final temperature depend on the duration of pumping action. With continuous pumping, cooling proceeds rapidly (15-20° per minute) and a very low temperature is reached. With intermittent pumping, cooling rate may be decreased, and any desired low temperature may be maintained within 0.5° after it is attained. For convenience of temperature regulation during observation, the pump motor switch should be next to the microscope. With this device we have reached temperatures at the slide surface near the object of −25° with a cooling-liquid temperature of −31° and a flow rate of 1 liter in 2 minutes.

Measurement of the temperature of the object is carried out with a thermocouple appressed by clamp d to the slide in the neighborhood of the object. The other thermocouple junction is maintained at a constant temperature of 0° (Dewar flask filled with melting ice). Temperatures were recorded by a galvanometer of high sensitivity (10^{-8}-10^{-9} A) with a small internal resistance. The temperature measured by the thermocouple does not correspond exactly to that of the object, which varies from place to place. Control temperature determinations have shown that in the center of the illuminated field (the warmest place), at an objective working distance of 1.5 mm, the actual temperature differs from that measured by the thermocouple by not more than 0.5-2° (depending on the rate and degree of cooling).

Objects to be frozen are placed on the slide in a drop of water or of paraffin oil and covered with a cover glass. When oil is used, and also to a lesser extent when water is used, objects are easily supercooled at 5-10°. In order to prevent this, the edge of the object is drawn out to the edge of the slide and brought in contact with the metal strip c. When the temperature of the slide has reached −1 or −2°, a drop of water is placed on the strip at the place of contact with the object. The water drop and the tip of the object freeze to the metal, ice forms in the object and then further through intercellular spaces and along the cut surface. With this procedure, if the object is in water, freezing begins at −1°. Freezing water somewhat impairs visibility of the object (in comparison with freezing in oil) if there is a cutinized surface uppermost (for example, the outer surface of epidermal cells). If, however, a cut surface is uppermost (i.e., walls of cells without a cutinized surface), then visibility is even better during freezing in water than in oil. The fact is that with freezing in oil, numerous ice crystals are formed on cell surfaces, and they grow rapidly as the temperature is lowered at the expense of water passing out of the cells and finally cover the cells, thus rendering observation impossible. If, however, freezing takes place in water, water passing out of the cells fuses with freezing water surrounding the object and freezes in the form of large, homogeneous, vitreous crystals through which the cells are visible. On cutinized surfaces ice crystals never form from water in the cells, and therefore cells mounted in oil are extremely visible during freezing.

Using the attachment described, we have made over a period of several years observations on the formation of intercellular and extracellular ice in various tissues and on its effect on cells; these observations have been documented with the MFN-1 photomicrographic attachment.

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*See English translation.

CURRENT EVENTS

THE EIGHTIETH BIRTHDAY OF PROFESSOR SERGEI LEONIDOVICH IVANOV

A. A. Prokof'ev

Translated from Fiziologiya Rastenii Vol. 7, No. 3, pp. 378-380, May-June, 1960

On May 23, 1960, eighty years will have passed since the birth of the great plant physiologist, biochemist, and doctor of biological sciences, Professor Sergi Leonidovich Ivanov. S. L. Ivanov completed (in 1903) the normal course of the Physics - Mathematics department of Moscow University. S. L. Ivanov began his professorial teaching activity in 1906 when he accepted a position as assistant at the Moscow Agricultural Institute. After this, S. L. Ivanov taught in various universities of the country the courses of botany, plant physiology and anatomy, and the chemistry of fats. His broad erudition, pedagogical talent and skillcarried the most complex problems to the students in a simple and at the same time clear form which invariably gave him the sympathy of the student audience.

In connection with the work, "Climatic Theory" (see below), S. L. Ivanov visited the Caucasus, Crimea, Central Urals, the Kola peninsula, Uzbekistan, Alta, the Far East, and also Finland, Switzerland, Italy, Holland, German, and other countries where he became acquainted with the flora and conditions of plant habitat. However, regardless of the institution and of the plants with which Sergei Leonidovich worked, all of his investigations conformed to a basic idea – the connection of biochemical processes in the plant world with Darwin's theory of evolution.

Taking oil plants as a basic object, S. L. Ivanov showed, as a result of a great deal of experimental work, the relationship of the oil-formation process in the plant seeds to a number of internal and external factors, and in particular to climate. We must remember that until the beginning of the twentieth century, when the scientific activity of S. L. Ivanov began, the area of plant oils and the problem of the biosynthesis of oils in plants was essentially untouched. Therefore, the detailed characteristics of the oils of different species of plants must have been preceded by the establishment of some sort of rules. The great work was done successfully by S. L. Ivanov and resulted in a number of publications by him. Of no less importance were the investigations which had the discovery of the rules of oil formation in the process of seed formation as a goal. The result of this work is a schematic formula of the oil formation process in plants, according to which saturated fatty acids are viewed as predecessors of the nonsaturated.

A great deal of attention was given by S. L. Ivanov to the development of a theory of the physiological-chemical characteristics of plants paralleled by the morphological features, and also a comparative study of the oils of species related in the systematic scheme. However, greatest renown came to S. L. Ivanov for the theory which he developed of the relationship of biochemical processes to climate ("Climatic Theory"). Basing it on a great deal of factual material, he introduced "the basic biochemical rule of the evolution of organic substance", which he expressed in the following manner.

"Each species has a capacity to develop under constant external conditions substances of a specific composition that suit its physiological-chemical characteristics.

"Each species shares its physiological-chemical characteristics with species that are in a close genetic connection with it; at the same time it possesses biochemical processes differentiating it from close species.

"With changes in the external conditions of the existence of physiological-chemical characteristics of the plants, changes for fatty oils follow the following geographical rule: Northern latitudes with a severe climate favor the formation in the oils of linolenic acid with three double bonds and oleic acid is formed in a minimum quantity; mild climates of the southern latitudes, on the other hand, favor the formation in the oils of oleic acid and a minimum quantity of linolenic and linoleic acids are formed."

"New physiological-chemical characteristics, which are in a basic relation to the ancestors, arise with the elimination of the genetic connection between species."

S. L. Ivanov proposed to separate his ideas on the rule of the geographical distribution of the organic substances of plants into a special scientific discipline, hulegeography. According to Sergei Leonidovich's idea, the subject of hulegeography is: "an explanation of the nature of specific substances of each large geographical region"; "an explanation of conditions leading to their synthesis"; "propagation of the specific substances"; "the possibility of transporting the producing plants to other countries". Although S. L. Ivanov's idea about the new discipline, hulegeography, has not obtained sufficient support among specialists, the ideas expressed by him on the connection between the physiological-chemical characteristics of plants and the climate of various geographical zones appeared to be very fruitful. Special investigations carried out with numerous species of plants in the various zones of the USSR and in foreign countries (especially the large contribution in introducing this in the former All-Union Institute of Horticulture) showed that a direct connection actually exists between the climatic conditions and the accumulation of reserve substances. For example, it was shown that a hot, dry climate supports a high protein content in the seeds and a damp, cool climate decreases it sharply. The accumulation in the seeds of nonnitrogen reserves, on the other hand, is strengthened under conditions of moderate temperatures and sufficient soil and atmospheric moisture. Apart from its purely scientific importance, the "climatic theory" of S. L. Ivanov has a very direct relationship to the practice of horticulture and, in particular, to the problem of a rational regionalization of agricultural crops.

S. L. Ivanov has completed eighty years. However, in spite of his dignified age, he is still full of strength and energy. Only recently S. L. Ivanov prepared a large work, "A Climatic Theory of the Formation of Organic Substances," for publication, in which the results of his many years of fruitful activity were presented. We wish this eminent scientist many more years of life, health, and creative success.

Close to 150 works were published by S. L. Ivanov. We present here a shortened list of the most significant words [for a more complete list, see the biographical-bibliographic Dictionary, "Russian Botanists" (1950) 3, p.415].

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A CONFERENCE DEDICATED TO THE 70th ANNIVERSARY OF THE BIRTH OF PROFESSOR D. A. SABININ

V. N. Zholkevich

Translated from Fiziologiya Rastenii Vol. 7, No. 3, p. 381, May-June, 1960

On March 30, 1960, in the House of Learning, USSR Academy of Sciences, there was a gathering of the scientific world dedicated to the 70th anniversary of the birth of a prominent Soviet plant physiologist and doctor of biological sciences, Professor Dmitrii Anatol'evich Sabinin.

D. A. Sabinin belonged to the group of those excellent scientists whose truly colossal erudition in conjunction with rare observation and remarkable ability to analyze facts made it possible to see significantly further than the majority of his contemporaries. Sometimes one is simply amazed at the accuracy of the conclusions that D. A. Sabinin reached, conclusions which fifteen years ago were supposed to have had inadequate and often even fantastic bases. History showed that D. A. Sabinin actually predicted the basic paths of the development of phytophysiology and the solution of a whole series of its cardinal problems many years in advance. Therefore, the more time passes, the clearer the great importance of D. A. Sabinin's work and his great activity as an experimenter and pedagogue for the future progress of science becomes. All of this makes us again and again return to an analysis of the creative path of D. A. Sabinin and the rich scientific inheritance left by him. Thus, for example, it was in 1955 that the 65th anniversary of the birth of D. A. Sabinin was noted in the House of Learning of the USSR Academy of Sciences, and in 1957 that the All-Union Botanical Society published one of the issues of the "Botany Journal" in Dmitrii Anatol'evich's memory, in connection with the sixth anniversary of his death.

Nearly 250 persons took part in the Jubilee Conference on March 30, 1960, including representatives of Moscow University, where D. A. Sabinin directed the Department of Plant Physiology, Institutes of the USSR Academy of Sciences including plant physiology, soils, botany, oceanology, biochemistry, and others, the Central Botanical Garden, Institutes of VASKhNIL, Timiryazev Agricultural Academy, various scientific-research organizations and universities in Moscow, Leningrad, and other cities of the USSR. Among those present were many students and colleagues of Dmitrii Anatol'evich – all his friends. There were also young physiologists who know Dmitrii Anatol'evich only through his works and through the tales of older comrades, never having been able to meet him personally.

In the introductory address, Professor A. V. Sokolov, noting the unusual fruitfulness of the D. A. Sabinin school, stressed that D. A. Sabinin was basically a new, progressive force in plant physiology, and is now, and will remain in the future, an ideal source for many physiologists.

A report on the life and scientific and pedagogical activity of D. A. Sabinin was given by O. V. Zalenskii, a candidate in the biological sciences. The speaker noted the great significance of such definitive works of Sabinin as "The Mineral Nutrition of Plants", "The Significance of the Root System in the Activity of Plants", and "The Physiological Bases of Plant Nutrition." These works, similar in depth of critical analysis, breadth of generalization, and elucidation of points not found in Russian or foreign literature, are the desk books of physiologists. Agronomists, and also biologists of other specialties, often turn to him. After this, reports containing new experimental data supporting the accuracy of D. A. Sabinin's ideas on the mineral nutrition of plants were presented by his students, biological science candidate F. F. Yukhimchuk ("The problem of nitrogen nutrition for legumes") and M. G. Zaitseva ("The effect of nitrogen-phosphate nutrition on the yield of summer wheat in the conditions of the extreme north"). An address by biological science candidate O. F. Tuevoi, "The cycle of phosphorus in plants," was included in the program of the conference; however it was not given because of illness.

Remembrances of D. A. Sabinin were given by corresponding member of the USSR Academy of Sciences L. A. Zenkevich, Professor A. V. Blagoveshchenskii, and biological science candidates A. K. Belousova and N. Z. Stankov.

All who came to the conference spoke of D. A. Sabinin with exceptional warmth and love. They characterized D. A. Sabinin as a daring scientific innovator, original thinker and researcher, as a talented pedagogue, a fascinating and sincere person who always remains in the memory of those who had the good fortune to study or work with him and of whom our native science is proud.

The conference expressed a desire for the early publishing of those works of Dmitrii Anatol'evich which have not been published yet, especially his monograph, "The physiology of plant development".

A display of D. A. Sabinin's work was organized in the House of Learning.

THE TRAVELING SESSION IN KAZAN OF THE ACADEMY OF SCIENCES USSR, DIVISION OF BIOLOGICAL SCIENCES,ON SUBJECTS RELATING TO THE WATER REGIMEN OF PLANTS

Translated from Fiziologiya Rastenii Vol. 7, No. 3, pp. 382-384, May-June, 1960

In Kazan during the period of March 2-7, 1960, the USSR Academy of Sciences' Division of Biological Sciences held a session which was devoted to the problems of water regimen in plants as related to their metabolism and productivity.

The basic network of scientific institutions of the USSR(where water regimen was under study) were invited to participate in the work of the session. There were reports about their work by physiologists from Moscow, Leningrad, Kazan, Tashkent, Stalinabad, Ufa, Kiev, Frunze, Alma-Ata, Baku, Tiraspol, Ulan-Udé, and other cities of our country. The total number of presented papers amounted to 62: on general problems dealing with the water regimen of plants, the water exchange of plants, the relationship of the water regimen with metabolism, and the ecology of water regimen.

The text of the resolution taken at the session is given here, as follows:

RESOLUTION

OF THE SCIENTIFIC SESSION,

DIVISION OF BIOLOGICAL SCIENCES,

IN KAZAN

The Communist Party and the Soviet government are devoting particularly great attention to the development of science in our country.

In the history making resolutions of the twenty-first convention of the USSR Communist Party, it is on record that during the seven-year period, conditions would be created for a still more rapid development of all branches of science, and the realization of important theoretical investigations, as well as great new scientific discoveries.

As designated in the ruling of the December 1959, Plenary Session of the Central Committee, USSR Communist Party, the most important problem of agricultural science consists of the development of theoretical investigations in the field of biology, physics, chemistry, and other adjacent sciences, which would promote a continuous rise of socialistic agriculture.

In the field of agriculture, the basic problem for the seven-year period is to achieve a level of production which would permit the fullest satisfaction of the need of the people for food and of industry for raw materials.

The increase in the productivity of plant industry is to be ensured by a complex of measures: improvement in agrotechnology, a wide use of fertilizers, improvement in seed quality, warfare against plant diseases and pests, etc. Of essential importance in increasing agricultural yields are the measures leading to the creation of an optimal water regimen for the plants, as well as to a higher resistance toward unfavorable external conditions.

Essential work in this direction has been performed by Soviet biologists. It was the objective of the present session to sum up some of the scientific results available, to bring up basic problems requiring further discussion and study, and to approbate practical recommendations. Kazan was chosen as the meeting place since it is one of the key centers devoted to the study of the water regimen in plants. The country's scientific institutions which are active in this field were invited to participate in the work of the session.

The main attention of the session was devoted to four major divisions:

I. General problems relating to the water regimen of plants.

II. Water exchange in plants.

III. Water regimen and metabolism in plants.

IV. Ecology of water regimen in plants.

I. General Problems of Water Regimen

The session took notice that the characteristic point of contemporary investigations on the water regimen consists of new aspects which consider the water regimen as a particular part of the basic character of living matter, namely, metabolism. This permits a look deeper into the essence of the water regimen, and permits a directed change.

On the basis of new concepts, the water regimen is studied in relation to the other metabolic aspects, to the energy production level, and to the influence of environmental factors. The various aspects of water regimen are thus considered in their close interrelationship to these.

As a result of investigations on water regimen in relationship to the other processes of life phenomena in plants, data were obtained which indicate a positive effect of high water saturation level on cells, an increase in these of colloidally bound water, a higher degree of hydration of the protoplasmic colloids and of the activity of the water (as reflected in photosynthesis, respiration, enzymatic activity, and growth), which all affect productivity of plants favorably.

The study pertaining to the effect of environmental conditions on the water regimen and the relationship of the latter to the other processes of plant life phenomena permitted approaching from a new angle the problem of plant resistance against unfavorable environmental conditions. This can be illustrated by the hardening of seeds before sowing, bringing about a higher hydrophilic condition, viscosity, and elasticity of the protoplasm, the activity of a series of enzymes, a higher intensity of photosynthesis and respiration, an elevation of the energizing level, and as a result a higher yield and productivity of the plants.

In studying the ontogenetic changes of the water regimen, much attention was thus being given to the changes in the water condition in the plant.

Work has been done on improving and refining the methods used in recording the water regimen of plants.

II. Water Exchange

The session took notice that definite accomplishments are on record in the field of water exchange in plants.

In contrast to the previous purely osmotic ideas, new concepts are being developed based on work in contemporary colloid, physical, and biological chemistry and on thermodynamic concepts of the state of water in plants and its activity.

It was shown that the entrance of water into plants, its movement and disbursement are dependent upon its activity status.

Investigation by means of heavy oxygen water H_2O^{18} showed that there exists in the plant cells a substantial amount of water which enters into exchange with difficulty, as well as some readily exchangeable water.

A relationship between the water regimen of plants and environmental conditions has been established. It was shown that the different aspects of water regimens are interrelated in different ways.

Alongside these achievements in the investigations of water exchange, there are also certain shortcomings. The new methods of investigation, especially biophysical, are being adapted at a slow pace. Some individual studies are mainly of descriptive nature, repeating well-known facts.

III. Water Regimen and Metabolism

The session states that the papers presented on this subject have established the existence of a varied relationship between the mineral regimen, mineral nutrition, and metabolism in plants. The submitted data testify to the possibility of an active influence on the water regimen and metabolism in plants for the purpose of raising their productivity and resistance. Especially effective and promising is the application of macro- and microquantities of elements, as well as the hardening of seeds before sowing.

The session acknowledges that the extension and deepening of investigations in the field of the relationship of water regimen and metabolism of plants would substantially advance not only the theory on the water regimen, but also the development of rational agrotechnical methods.

Aside from the general achievements in the field of studies on the water regimen of plants, little attention is being devoted to the relationship between the water regimen and such physiological processes as photosynthesis, respiration, and growth. There are practically no publications dealing with the study of the water balance in plants. Only a few links in metabolism have been touched upon by biochemical investigations, which does not permit a deep penetration into the essence of the relationship between water regimen and metabolism.

There is an urgent need to improve the methods for the investigation of the various fractions of water and of the other aspects of the water regimen.

As a rule, there is a lack of variational-statistical treatment of the numerical experimental material.

IV. Ecology of Water Regimen in Plants

The session notes that the study of the water regimen in plants from an ecological-physiological point of view represents one of the important and promising aspects of water regimen physiology.

There has accumulated on this problem a large volume of factual material which characterizes the physiological peculiarities of plants growing under various ecological-geographical conditions. This is of great importance in elucidating the ways plants adjust to environmental conditions.

There are a number of publications dealing with the influence of single environmental factors upon the ratio of the various forms of water in the plant, on the transpiration intensity, the water-retaining capacity of the leaves, etc.

However, the investigations on the problem of ecology of the plant water regimen are, in general, of a quite scattered character. In a number of papers, the study on the influence of environmental factors upon the water regimen of plants is confined to the elucidation of these factors only with respect to single phases of the water regimen. This partially explains the absence of a deep analysis of causes and of a broader ecological interpretation of the basic phenomena of the plant water regimen.

The session wishes to point out the substantial contributions made by the physiologists of the Kazan Section of the USSR Academy of Sciences and of Kazan University, headed by Prof. A. M. Alekseev, in the study of water regimen in plants. Their concepts, dealing with the forms, the mechanism of water intake (and its

movement in plants), the relationship of water regimen with metabolism (especially in relation to mineral nutrition), are factual, original, and have perspective. One should also note the high methodological level of work, the extensive utilization of physical and chemical data, and the invariable subsequent treatment of the experimental data by means of the variational-statistical method. The results from the work of the Kazan group of physiologists are of great vaue in lifting the general theoretical and methodological level of water regimen investigations.

The session considers it highly important to deepen and broaden further the work of the plant physiology laboratory of the Kazan section, USSR Academy of Sciences, and of the plant physiology chair at Kazan University.

The session suggests to Prof. A.M. Alekseev and to other Kazan physiologists to prepare for publication a monograph on the principal basic problems of water regimen which would encompass all the most recent achievements in this field of knowledge.

The session deems it necessary to concentrate the attention in forthcoming experiments on the development of the following problems.

1. Investigation on the status of water in the plant cell, involving a deepened study on the swelling of high-molecular substances, especially proteins and nucleoproteins.

2. A study on the distribution of water in various parts and organules of cells, using water isotopes.

3. A deepened study on the interrelationship between various metabolic fragments and the water regimen in plants, using the newest technical methods of investigation. Special attention is to be given to the interrelationship of the water regimen with photosynthesis, respiration, activity of various enzymes, oxidation-reduction conditions in the cells, and the general energy level in the plant, as well as the relationship between the water regimen and metabolism in the roots.

4. A deepened study on the specific influence of individual macro- and microelements on the metabolism and water regimen in plants.

5. A more detailed investigation on the ecology of water regimen in plants, embracing as many wild and cultured varieties as possible.

6. Investigation on the morphological-anatomical structure of plants under a variety of ecological settings and water regimens.

7. Further development of the methods leading to an increase of drought resistance in plants, based on the study of peculiarities in metabolism and water regimen in plants under drought conditions. Special attention should be given to further development of the quite promising method of seed hardening before sowing, in combination with the effect of microelements.

8. A study of the effect of low and variable temperatures, as well as other factors, on metabolism and water regimen, aiming at a higher frost and cold resistance of cultured plants

9. Working out new methods of investigating the water regimen of plants by making use of recent achievements in physics and chemistry (the method of proton resonance, heavy water, etc.).

The session wishes to emphasize once more that the final goal of all investigations on the water regimen should be an increase in the harvest yield of agricultural plants and their resistance to unfavorable environmental conditions.

On the Publication of the Session Proceedings

Taking in consideration the great importance of the present session, which has summarized the results of many years' work by Soviet physiologists on the water regimen of plants, with further investigations having been mapped, the session recommends the issue by the Division of Biological Sciences, via the USSR Academy of Sciences publishing branch, a symposium of the papers presented at the meetingsof the traveling session, a total of 30 signatures.

ERRATA

Vol. 6, No. 5

Page	Line	Reads	Should read
531	10	transmission	translocation
533	Table 3 left-hand heading	NO_3^- assimilated in darkness without CO_2	NO_3^- assimilated in light without CO_2
554	7	equality	equilibrium
561	32 from bottom	the isotope separates	isotopic dilution
565	23 from bottom	covering	shell
577	16 from bottom	di- and tricarbonic	di- and tricarboxylic
598	Table 4 (6 times)	{	{c e
610	26 from bottom	. . . volume was 5.0 ml of a glycolic acid . . .	. . . volume was 5.0 ml. In the third series it consisted of 4.0 ml of a glycolic acid . . .
649	5	ionized	ionizing

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685	Table 1	Ratio chlorotic to greening	Ratio greening to chlorotic
701	22 from bottom	oxidizing	oxidative
711	3 from bottom	cation	cation exchanger
712	5	cation	cation exchanger
712	7-8	based on . . . water	based on the extraction of organic acids from an acid solution into ether
712	16	levels	rates of movement
712	19	distillation	separation
712	8 from bottom	face meter	end-window counter
712	7 from bottom	efficiency of the calculation	counting efficiency
715	23 from bottom	oxypyruvic	hydroxypyruvic
715	16 from bottom	di- and tricarbonic	di- and tricarboxylic
716	12-13	the development . . . fact that	the appearance of the label in the citric, malic, succinic, and fumaric acids shows that . . .
762	21	ionized	ionizing

ABBREVIATIONS MOST FREQUENTLY ENCOUNTERED IN RUSSIAN BIO-SCIENCES LITERATURE

Abbreviation (Transliterated)	Significance
AMN SSSR	Academy of Medical Sciences, USSR
AN SSSR	Academy of Sciences, USSR
BIN	Biological Institute, Botanical Institute
FTI	Institute of Physiotherapy
GONTI	State United Sci-Tech Press
GOST	All Union State Standard
GRRRI	State Roentgenology, Radiology, and Cancer Institute
GTTI	State Technical and Theoretical Literature Press
GU	State University
I Kh N	Scientific Research Institute of Surgical Neuropathology
IL (IIL)	Foreign Literature Press
IONKh	Inst. Gen. and Inorganic Chemistry (N. S. Kurnakov)
IP	Soil Science Inst. (Acad. Sci. USSR)
ISN (Izd. Sov. Nauk)	Soviet Science Press
Izd.	Press
LEM	Laboratory for experimental morphogenesis
LENDVI	Leningrad Inst. of Dermatology and Venereology
LEO	Laboratory of Experimental Zoology
LIKhT	Leningrad Surgical Institute for Tuberculosis and Bone and Joint Diseases
LIPZ	Leningrad Inst. for Study of Occupational Diseases
LIPK	Leningrad Blood Transfusion Institute
Medgiz	State Medical Literature Press
MOPISh	Moscow Society of Apiculture and Sericulture
MVI	Moscow Veterinary Institute
MZdrav	Ministry of Health
MZI	Moscow Zootechnical Institute
LOKhO	Leningrad Society of Orthopedic Surgeons
NIIZ	Scientific Research Institute of Zoology
NINKhI	Scientific Research Institute of Neurosurgery
NIU	Scientific Institute for Fertilizers
NIUIF	Scientific Research Institute of Fertilizers and Insecticides
NIVI	Veterinary Scientific Research Institute
ONTI	United Sci.-Tech. Press
OTI	Division of Technical Information
RBO	Russian Botanical Society
ROP	Russian Society of Pathologists
SANIIRI	Central Asia Scientific Research Institute of Irrigation
SANIISh	Central Asia Scientific Research Institute of Sericulture
TsNII	All-Union Central Scientific Research Institute
TsNTL	Central Scientific and Technical Laboratory
VASKhNIL	All-Union Academy of Agricultural Sciences
VIG	All-Union Institute of Helminthology
VIEM	All-Union Institute of Experimental Medicine
VIR	All-Union Institute of Plant Cultivation
VIUAA	All-Union Institute of Fertilizers, Soil Science, and Agricultural Engineering
VIZR	All-Union Institute of Medical and Pharmaceutical Herbs
VNIRO	All-Union Scientific Institute of Fishing and Oceanography
ZIN	Zoological Inst. (Acad. Sci. USSR)

Note: Abbreviations not on this list and not explained in the translation have been transliterated, no further information about their significance being available to us. - Publisher.

RUSSIAN JOURNALS FREQUENTLY CITED
[Biological Sciences]

Abbreviation*	Journal*	Translation
Agrobiol.	Agrobiologiya	Agrobiology
Akusherstvo i Ginekol.	Akusherstvo i Ginekologiya	Obstetrics and Gynecology
Antibiotiki	Antibiotiki	Antibiotics
Aptechnoe Delo	Aptechnoe Delo	Pharmaceutical Transactions
Arkh. Anat. Gistol. i Émbriol.	Arkhiv Anatomii Gistologii i Émbriologii	Archives of Antomy, Histology, and Embryology
Arkh. Biol. Nauk SSSR	Arkhiv Biologicheskikh Nauk SSSR	Archives of Biological Science USSR
Arkh. Patol.	Arkhiv Patologii	Archives of Pathology
Biofizika	Biofizika	Biophysics
Biokhimiya	Biokhimiya	Biochemistry
Biokhim. Plodov i Ovoshchei	Biokhimiya Plodov i Ovoshchei	Biochemistry of Fruits and Vegetables
Bot. Zhur.	Botanicheskii Zhurnal	Journal of Botany
Byull. Éksptl. Biol. i Med.	Byulleten Éksperimentalnoi Biologii i Meditsiny	Bulletin of Experimental Biology and Medicine
Byull. Moskov. Obshchestva Ispytatelei Prirody, Otdel Biol.	Byulleten Moskovskogo Obshchestva Ispytatelei Prirody, Otdel Biologicheskii	Bulletin of the Moscow Naturalists Society, Division of Biology
Doklady Akad. Nauk SSSR	Doklady Akademii Nauk SSSR	Proceedings of the Academy of Sciences USSR
Éksptl. Khirurg.	Éksperimentalnaya Khirurgiya	Experimental Surgery
Farmakol. i Toksikol.	Farmakologiya i Toksikologiya	Pharmacology and Toxicology
Farmatsiya	Farmatsiya	Pharmacy
Fiziol. Rastenii	Fiziologiya Rastenii	Plant Physiology
Fiziol. Zhur. SSSR	Fiziologicheskii Zhurnal SSSR im. I. M. Sechenova	I. M. Sechenov Physiology Journal USSR
Gigiena i Sanit.	Gigiena i Sanitariya	Hygiene and Sanitation
Izvest. Akad. Nauk SSSR, Ser. Biol.	Izvestiya Akademii Nauk SSSR, Seriya Biologicheskaya	Bulletin of the Academy of Sciences USSR, Biology Series
Izvest. Tikhookeanskogo N. I. Inst. Rybnogo Khoz. i Okeanog.	Investiya Tikhookeanskogo N. I. Instituta Rybnogo Khozyaistva i Okeanografii	Bulletin of the Pacific Ocean Scientific Institute of Fisheries and Oceanography
Khirurgiya	Khirurgiya	Surgery
Klin. Med.	Klinicheskaya Meditsina	Clinical Medicine
Lab. Delo	Laboratornoe Delo (po Voprosam Meditsiny)	Laboratory Work (on Medical Problems)
Med. Parazitol.	Meditsinskaya Parazitologiya i Parazitarnye Bolezni	Medical Parasitology and Parasitic Diseases
Med. Radiol.	Meditsinskaya Radiologiya	Medical Radiology
Med. Zhur. Ukrain.	Medichnii Zhurnal Ukrainskii	Ukrainian Medical Journal
Mikrobiologiya	Mikrobiologiya	Microbiology
Mikrobiol. Zhur.	Mikrobiologichnii Zhurnal	Microbiology Journal
Nevropatol., Psikhyat. i Psikhogig.	Nevropatologiya, Psikhyatriya i Psikhogigiena	Neuropathology, Psychiatry and Psychohygiene
Ortoped., Travmatol. i Protez.	Ortopediya, Travmatologiya i Protezirovanie	Orthopedics, Traumatology and Prosthetics
Parazitol. Sbornik	Parazitologicheskii Sbornik	Parasitology Collection
Pediatriya	Pediatriya	Pediatrics
Pochvovedenie	Pochvovedenie	Soil Science
Priroda	Priroda	Nature
Problemy Éndokrinol. i Gormonoterap.	Problemy Endokrinologii i Gormonoterapii	Problems of Endocrinology and Hormone Therapy
Problemy Gematol.	Problemy Gematologii i Perelivaniya Krovi	Problems of Hematology and Blood Transfusion
Problemy Tuberk.	Problemy Tuberkuleza	Problems of Tuberculosis
Sovet. Med.	Sovetskaya Meditsina	Soviet Medicine
Sovet. Vrachebny Zhur.	Sovetskii Vrachebnyi Zhurnal	Soviet Physicians Journal
Stomatologiya	Stomatologiya	Stomatology

*BRITISH-AMERICAN TRANSLITERATION SYSTEM.

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Abbreviation	Journal	Translation
Terap. Arkh.	Terapevticheskii Arkhiv	Therapeutic Archives
Trudy Gelmint. Lab.	Trudy Gelmintologicheskoi Laboratoriya	Transactions of the Helminthology Laboratory
Trudy Inst. Genet.	Trudy Instituta Genetiki	Transactions of the Institute of Genetics
Trudy Inst. Gidrobiol.	Trudy Instituta Gidrobiologiya	Transactions of the Institute of Hydrobiology
Trudy Inst. Mikrobiol.	Trudy Instituta Mikrobiologiya	Transactions of the Institute of Microbiology
Trudy Inst. Okean.	Trudy Instituta Okeanologiya, Akademii Nauk SSSR	Transactions of the Institute of Oceanology, Academy of Sciences, USSR
Trudy Leningrad Obshchestva Estestvoisp.	Trudy Leningrad Obshchestva Estestvoispytatelei	Transactions of the Leningrad Society of Naturalists
Trudy Vsesoyuz. Gidrobiol. Obshchestva	Trudy Vsesoyuznogo Gidrobiologicheskogo Obshchestva	Transactions of the All-Union Hydrobiological Society
Trudy Vsesoyuz. Inst. Ekspt. Med.	Trudy Vsesoyuznogo Instituta Eksperimentalnoi Meditsiny	Transactions of the All-Union Institute of Experimental Medicine
Ukrain. Biokhim. Zhur.	Ukrainskii Biokhimichnii Zhurnal	Ukrainian Biochemical Journal
Urologiya	Urologiya	Urology
Uspekhi Biokhimiya	Uspekhi Biokhimiya	Progress in Biochemistry
Uspekhi Sovremennoi Biol.	Uspekhi Sovremennoi Biologiya	Progress in Contemporary Biology
Vestnik Akad. Med. Nauk SSSR	Vestnik Akademii Meditsinskikh Nauk SSSR	Bulletin of the Academy of Medical Science USSR
Vestnik Khirurg. im. Grekova	Vestnik Khirurgii imeni Grekova	Grekov Bulletin of Surgery
Vestnik Leningrad. Univ. Ser. Biol.	Vestnik Leningradskogo Universiteta, Seriya Biologii	Journal of the Leningrad Univ., Biology Series
Vestnik Moskov. Univ., Ser. Biol. i Pochvov.	Vestnik Moskovskogo Universitera, Seriya Biologii i Pochvovedeniya	Bulletin of the Moscow University, Biology and Soil Science Series
Vestnik Oftalmol.	Vestnik Oftalmologii	Bulletin of Ophthalmology
Vestnik Oto-rino-laringol.	Vestnik Oto-rino-laringologiya	Bulletin of Otorhinolaryngology
Vestnik Rentgenol. i Radiol.	Vestnik Rentgenologii i Radiologii	Bulletin of Roentgenology and Radiology
Vestnik Venerol. i Dermatol.	Vestnik Venerologii i Dermatologii	Bulletin of Venereology and Dermatology
Veterinariya	Veterinariya	Veterinary Science
Vinodelie i Vinogradarstvo	Vinodelie i Vinogradarstvo SSSR	Wine-Making and Viticulture
Voprosy Klin.	Voprosy Klinicheskie	Clinical Problems
Voprosy Med. Khim.	Voprosy Meditsinskoi Khimii	Problems of Medical Chemistry
Voprosy Med. Virusol.	Voprosy Meditsinskoi Virusologii	Problems of Medical Virology
Voprosy Neirokhirurg.	Voprosy Neirokhirugii	Problems of Neurosurgery
Voprosy Onkol.	Voprosy Onkologii	Problems of Oncology
Voprosy Pitaniya	Voprosy Pitaniya	Problems of Nutrition
Voprosy Psikhologii	Voprosy Psikhologii	Problems of Psychology
Voprosy Virusologii	Voprosy Virusologii	Problems of Virology
Vrachebnoe Delo	Vrachebnoe Delo	Medical Profession
Zav. Lab.	Zavodskaya Laboratoriya	Factory Laboratory
Zhur. Mikrobiol., Epidemiol. i Immunobiol.	Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii	Journal of Microbiology, Epidemiology, and Immunobiology
Zhur. Nevropatol. i Psikhiat.	Zhurnal Nevropatologii i Psikhiatrii imeni S. S. Korsakov	S. S. Korsakov Journal of Neuropathology and Psychiatry
Zhur. Obshchei Biol.	Zhurnal Obshchei Biologiya	Journal of General Biology
Zhur. Vysshei Nerv. Deyatel.	Zhurnal Vysshei Nervnoi Deyatelnosti imeni I. P. Pavlova	I. P. Pavlov Journal of Higher Nervous Activity
Zool. Zhur.	Zoologicheskii Zhurnal	Journal of Zoology